

Parasites of Jellyfish in Eastern Australia

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CHAPTER 1 GENERAL INTRODUCTION:



The isopod *Cymodoce gaimardii* on the jellyfish *Catostylus mosaicus*
(bell diameter ~ 250 mm)

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Abstract

Parasites are a major component of the Earth's biota, yet are often overlooked and their importance underestimated. They affect nearly all organisms and can potentially regulate the populations of their hosts. Jellyfish are abundant members of the zooplankton community and are renowned for their ability to form large blooms. Jellyfish host a large diversity of parasites. Some of these parasites (e.g. hyperiid amphipods and parasitic anemones) use jellyfish as the sole host in their life cycle, whilst others (e.g. digenean trematodes and cestodes) use jellyfish as an intermediate host between other host organisms. There have been few ecological studies of jellyfish parasites (for example of spatial and temporal variation in parasite-host relationships) relative to other aspects of jellyfish biology. This may be partly attributable to the very small size of some parasites (e.g. digenean trematodes) and also to difficulties such as identifying larval parasite forms. However, with the advent of new technologies, such as molecular identification, and with diligent examination, these difficulties are able to be overcome. This thesis aims to identify jellyfish parasites using molecular and morphological techniques and elucidate their life cycles, examine spatial and temporal variation in jellyfish parasites and investigate the diversity and host specificity of the parasites of jellyfish in eastern Australia.

Jellyfish are consumed by a range of vertebrate predators and act as intermediate hosts for some parasites, including digenean trematodes. Identification of digeneans is based upon the reproductive organs of adults. These organs are absent in the metacercariae stage which infects jellyfish. DNA sequences of the ribosomal ITS2 spacer can be analysed to differentiate digenean species, thus, sequences of identified adults can be matched to those of unknown metacercariae. In Chapter Two, this technique was combined with morphological analysis to identify two lepecreadiid digenean species from jellyfish and fish hosts. Three species of jellyfish were captured within Port Phillip Bay, Victoria and three species of fish, known to feed upon jellyfish, were obtained from Moreton Bay, Queensland and Port Phillip Bay and Portland in Victoria. The location inside the jellyfish, intensity (the number of parasites per host), and prevalence (the percentage of hosts that are infected) of the digeneans were measured. The digeneans were distributed throughout most parts of the jellyfish. *Opechona bacillaris* parasitised the scyphozoan jellyfish *Aequorea eurodina* and the scombrid fish *Scomber australasicus*. *Cephalolepidapedon warehou* occurred in the scyphozoans *Pseudorhiza haeckeli* and *Cyanea annaskala* and the centrolophid fish *Seriotelella brama* and *Seriotelella punctata*. Intensities ranged from four to 96 in the jellyfish, and one to 30 in the fish. For each

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digenean species, the ITS2 sequences from mature adults in the fishes matched those from the metacercariae parasitising the jellyfish. This was the first record of a larval stage of *C. warehou* and was the first use of DNA sequencing to identify digenean metacercariae from jellyfish. Three new host records were obtained for *C. warehou* and one for *O. bacillaris*. Morphological and ecological data from this and other studies suggest that jellyfish act as second intermediate hosts for these digenean species.

Spatial variation in the prevalence and intensity of digenean and cestode endoparasites of the upside-down jellyfish *Cassiopea* sp. was determined in Chapter Three. Jellyfish and their endoparasites were sampled at four locations over approximately 13 degrees of latitude: two locations (Lizard Island and Vlasoff Reef) were on the Great Barrier Reef and two locations (Lake Magellan and Crab Island) were in southeast Queensland. Molecular and morphological techniques were used to differentiate and identify endoparasites. The endoparasites found included one larval cestode morphotype, 15 encysted digenean metacercariae morphotypes and six unencysted digenean metacercariae. Communities of endoparasites differed between the four locations and only three morphotypes were present in more than one location. The greatest diversity of endoparasites occurred at Lizard Island (17 morphotypes) and Vlasoff Reef (six morphotypes) and diversity was low at both Lake Magellan and Crab Island (two morphotypes each). ITS2 sequences were obtained for six specimens of digeneans. Sequencing allowed the identification of one digenean morphotype as *Pseudopisthgonoporus vitellosus*; the likely placement of one morphotype within the family Apocreadiidae; and the confirmation of one species at two locations.

The diversity of digeneans parasitising jellyfish at Lizard Island was investigated in Chapter Four. Three species of hydromedusae (*Aequorea australis*, *Malagazzia carolinae* and *Olindias singularis*) and two species of ctenophore (*Bolinopsis* sp. and *Beroe* sp.) were dissected and the digeneans removed. The digeneans were sequenced for ITS2 and LSU DNA regions and identified using morphological and phylogenetic analyses. Sixteen species of digenean were differentiated from three superfamilies (Lepocreadioidea, Hemiuroidea and Haploporoidea). One digenean was identified to species (*Opechona bacillaris*), and two to genus level (*Lecithocladium* sp. and *Isorchis*). The prevalence of infection by total digeneans ranged from 6.7% to 100%, and mean intensity varied between host species. There was evidence of host specificity and within host site specificity.

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Chapter Five examined temporal variation in the abundances of parasites and associates of the scyphozoan jellyfish *Catostylus mosaicus* over a two year period. Jellyfish were captured from Port Phillip Bay, Victoria every six weeks, dissected and inspected for symbionts. Parasitic amphipods (*Hyperia gaudichaudii*) and anemones (*Peachia hilli*) were observed, as were an associated sphaeromatid isopod (*Cymodoce gaimardii*) and juvenile fish (*Seriolella brama* and monocanthids including *Acanthaluteres* sp.). There was temporal variation in the presence and abundances of the symbionts. Eggs and juvenile *H. gaudichaudii* were endoparasitic within the jellyfish, and juveniles and mature males and females attached to the outer surfaces. The highest abundances of *H. gaudichaudii* occurred prior to the disappearance of jellyfish from the sampling area in September 2008. No *H. gaudichaudii* were present in six-weekly plankton tows, indicating the adults are unlikely to have a pelagic life style.

The use of a combined morphological and molecular approach in this research was novel and provided new insights about the diversity of parasites hosted by jellyfish, the life cycles of parasites and the potential importance of jellyfish as intermediate hosts of fish parasites. Three digeneans were identified to species level, 35 additional morphotypes were distinguished and new sequences were obtained for nine species. Five new host records were obtained for the three identified digeneans. For two of these species, intermediate hosts were discovered for the first time, adding to the knowledge of their life cycles. For the first time, larval stages of digeneans belonging to the Family Atractotrematidae were also identified. This research is among the few studies of cestodes or sphaeromatid isopods associating with jellyfish. New host jellyfish species were recorded for a larval cestode and the isopod *Cymodoce gaimardii*. Ecological studies of jellyfish parasites are uncommon. Moreover, spatial variation in endoparasites of *Cassiopea* sp.; the diversity of digeneans parasitising a range of jellyfish in tropical waters; and temporal variation in relationships between *Catostylus mosaicus* and its associates in Port Phillip Bay were investigated. The high diversity of jellyfish parasites and identification of new jellyfish hosts indicates a greater importance of jellyfish as hosts than previously recognised. This research has added to the knowledge of jellyfish parasites and will provide a valuable contribution to further experimental and ecological studies.

Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.



Joanna Browne

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Dedication

I would like to dedicate this thesis to Dad and to Lucie Leonard. Dad, you fostered my love of the ocean and books. Your traits of meticulousness and quiet determination got me through this. Lucie, you were with me from primary school to university and beyond. Your strength and positive outlook until the end will always be an inspiration to me.

You both shaped my life and left it far too soon.

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Glossary

Term	Definition
Abundance	"the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected" (Bush et al. 1997)
Accidental host	a host which is infected by a parasite but which does not contribute to the life cycle (for example, only immature <i>Opechona bacillaris</i> have been found in boarfish <i>Capros aper</i> and the lumpsucker <i>Cyclopterus lumpus</i> suggesting the digenean is unable to metamorphose and complete its life cycle in these fish (Nicoll 1910; Nicoll 1914))
Cercaria	larva of the sexual adult of a digenean, usually with a tail for swimming
Definitive host	host in which in which the parasite matures and reproduces sexually
Ectoparasite	a parasite on the surface of a host
Endoparasite	a parasite in the interior of a host
Gelatinous zooplankton	organisms including jellyfish, chaetognaths, pteropods, heteropods, appendicularians, salps, doliolids, and pyrosomes
Intensity	"the number of individuals of a particular parasite species in a single infected host" Bush et al.(1997)
Intermediate host	a host which is necessary for the life cycle of a parasite to be completed. It may be needed for the parasite to metamorphose in or for parasite to be transmitted to the next host (cercariae develop in the first intermediate host, whilst metacercariae develop in the second intermediate host)
Jellyfish	organisms including scyphomedusae, hydromedusae, cubomedusae, siphonophores and planktonic ctenophores
Jellyfish digenean	any digenean which parasitises jellyfish

Cont.

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Term	Definition
Jellyfish parasite	parasites which use jellyfish as hosts
Metacercaria	intermediate resting stage between a cercaria and sexual adult of a digenean
Miracidium	motile ciliated larvae digenean larvae
Parasite	“an organism living in or on another organism, the host-feeding on it, showing some degree of structural adaptation to it, and causing it some harm” (Poulin 2007)
Paratenic host	a host that is used in the life cycle of a parasite, but is not compulsory for the completion of the life cycle (may also be referred to as a transport host)
Planula	free-swimming larval form of a cnidarian
Plerocercoid	developmental cestode stage which develops within a second intermediate host
Polyp	asexually reproducing generation of a cnidarian
Prevalence	“the number of hosts infected with 1 or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species” (Bush et al. 1997)

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1 General Introduction

1.1 Parasites

It is likely that there are as many, if not more parasitic species on Earth as non-parasitic species (Price 1980; Køie 1991; Windsor 1995; Windsor 1998), yet parasites are often overlooked in biodiversity and ecological studies and their importance is underestimated (Marcogliese 2004; Poulin 2004). They play important roles in ecosystems by affecting fecundity of their hosts (e.g. Køie 1975), influencing food web dynamics (Lafferty et al. 2006), affecting mate choice and sex ratios (Minchella and Scott 1991) and influencing rates of predation upon their hosts (Lafferty and Morris 1996). Nearly all organisms spend energy to avoid parasites or cope with their effects (Littlewood 2005).

Marine parasites can be useful for studying ecological processes as they inhabit small and well defined habitats in a comparatively constant environment (e.g. studies of competition and habitat partitioning, Rohde 1976). They are frequently used as biological markers in studies of host populations and migrations (Rohde 2001; Niklitschek et al. 2010; Carballo et al. 2012) and are potential biological control agents against invasive species (Torchin et al. 2003). Increasingly, parasites are being recognised as potentially useful indicators of pollutants and ecosystem health (Lafferty 1997; Marcogliese 2005b).

The distribution and population dynamics of parasites depend on their hosts, while the growth and reproductive rates of hosts can be influenced by parasites. Mathematical models suggest that parasites regulate populations of their hosts (e.g. Roberts and Dobson 1995). Parasites have occasionally been proposed as a potential cause of population crashes, for example, in fish (Heins et al. 2010). Factors which affect the impact of a parasite include their mode of transmission and host specificity (Poulin 2007).

1.2 Jellyfish and their parasites

Jellyfish comprise a large component of marine zooplankton and are renowned for their ability to form spectacular population blooms that comprise a significant proportion of the pelagic consumer biomass (e.g. Mianzan and Guerrero 2000; Pitt and Kingsford 2003). The 'boom and bust' nature of jellyfish populations means that they can have intense, localised effects on the ecology (e.g. Pitt et al. 2007; Pitt et al. 2009) and nutrient dynamics (Pitt et al. 2009) of the systems they inhabit. Since the early 2000s there has been considerable concern that jellyfish populations have been increasing globally (e.g.

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Brodeur et al. 1999; Shiganova and Bulgakova 2000). A recent meta-analysis that utilised all long-term (>10 years) time series data on jellyfish available from across the world, however, found insufficient evidence to conclude that jellyfish populations were rising globally (Condon et al. 2013). The analysis did, however, identify regions where jellyfish populations were increasing (e.g. Sea of Japan, northern Adriatic Sea) as well as regions where jellyfish populations were in decline (e.g. upper North Sea, northwest Bering Sea). Some species of jellyfish are also highly invasive (e.g. the ctenophore *Mnemiopsis leidyi*) and the spread of invasive species of jellyfish is changing the distribution of many taxa (Graham and Bayha 2007).

Jellyfish host many types of parasites including sea anemones, digenean trematodes, nematodes, copepods, hyperiid amphipods, and barnacles (Table 1-1). For some parasites, jellyfish are the sole host for the duration of the parasite's life cycle (e.g. hyperiid amphipods, Dittrich 1988) whilst for others they are an intermediate host between other host organisms (e.g. digenean trematodes, Køie 1975). In some cases, jellyfish are intermediate hosts for parasites which ultimately parasitise commercially important fish species. For example, the ctenophore *Pleurobrachia pileus* harbours the nematode *Hysterothylacium aduncum*, a parasite of farmed sea trout. *Hysterothylacium aduncum* can kill larvae of commercially important fish such as herring (Balbuena et al. 2000) and halibut (Bristow 1990 cited in Skovgaard et al. 2011). Blooms of jellyfish are likely to be particularly susceptible to parasitism as host densities are positively correlated to parasite abundances (Arneberg et al. 1998) and host population size is a determinant of parasite infection (e.g. Bagge et al. 2004). Consequently, changes in abundances and distributions of jellyfish that act as intermediate hosts could have implications for commercial fish stocks.

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Table 1-1 Examples of parasites from jellyfish

Parasite		Jellyfish		Author and Year
Group	Species	Group	Species	
Gymnamoebae	? <i>Flabellula</i> sp.	Ctenophore	<i>Mnemiopsis mccradyi</i>	Moss et al. 2001
Protoodinid dinoflagellate	similar to <i>Protoodinium chattoni</i>	Ctenophore	<i>Mnemiopsis mccradyi</i>	Moss et al. 2001
Nematode	<i>Hysterothylacium aduncum</i>	Ctenophore	<i>Pleurobrachia pileus</i>	Mutlu and Bingel 1999
Digenean trematode	<i>Cephalolepidapedon saba</i>	Scyphozoan	<i>Aurelia</i> sp.	Ohtsuka et al. 2010
Cestode	unknown	Scyphozoan	<i>Periphylla periphylla</i>	Phillips and Levin 1973
Sea anemone	<i>Edwardsiella leidy</i>	Ctenophore	<i>Beroe ovata</i>	Reitzel et al. 2007
Sea anemone	<i>Edwardsiella leidy</i>	Ctenophore	<i>Mnemiopsis leidy</i>	Reitzel et al. 2007
Sea anemone	<i>Peachia hilli</i>	Scyphozoan	<i>Catostylus mosaicus</i>	Badham 1917
Parasitic narcomedusan larvae	Unknown	Narcomedusan	<i>Solmissus incisa</i>	Osborn 2000
Gammarid amphipod	<i>Metopa alderi</i>	Hydromedusan	<i>Tima bairdii</i>	Vader 1972
Hyperiid amphipod	<i>Lestrigonus bengalensis</i>	Siphonophore	<i>Diphyes bojani</i>	De Lima and Valentin 2001
Barnacle	<i>Alepas pacifica</i>	Scyphozoan	<i>Diplulmaris malayensis</i>	Pagès 2000
Pycnogonid	<i>Pallenopsis tritonis</i>	Anthomedusan	<i>Pandea rubra</i>	Pagès et al. 2007
Isopod (larvae)	Miktoniscus larvae of an epicaridean	Ctenophore	<i>Pleurobrachia pileus</i>	Yip 1984

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1.3 Life cycles of parasites

Hyperiid amphipods (review: Laval 1980), parasitic anemones (e.g. Reitzel et al. 2007) and digeneans (e.g. K  ie 1991) are three of the most common taxa that parasitise jellyfish. Most hyperiid amphipods and parasitic anemones have direct life cycles, requiring only one host to complete their life cycle. Digeneans, however, have an indirect life cycle, requiring multiple hosts for their development, with jellyfish acting as an intermediate host for some species. Less common than digeneans, some cestodes also use jellyfish as intermediate hosts. Some parasites have a wide range of potential hosts (e.g. the amphipod *Hyperia galba* has been recorded from eight species of scyphozoan, (Bowman et al. 1963; Dittrich 1988; Ohtsuka et al. 2010; Fleming et al. 2014), while others are recorded from just one genus (e.g. Laval 1980). Parasites found in multiple hosts may be better adapted to one host than others. For example, the parasitic anemone, *Edwardsiella lineata* is found in two types of ctenophore (Table 1-1) yet develops more quickly and successfully in one of these species (Reitzel et al. 2007).

1.3.1 Single host life cycles

1.3.1.1 Hyperiid amphipods

Most hyperiid amphipods depend on gelatinous zooplankton for at least part of their life cycle (Arai 2005a). In a typical life cycle, a female amphipod broods eggs and young in a specially developed pouch. Once the juvenile stage is reached, the female deposits the young onto a gelatinous host (Laval 1980). She may swim between hosts, depositing larvae onto more than one host (Laval 1980). The young stays in or on the host and feeds on its tissues (Laval 1980) as it grows. Once the hyperiids have developed into adults they may use their hosts as a food source, a resting spot, shelter or transport (L  tzen 2005; Fleming et al. 2014). Females often remain closely associated with the host, while males are often free-swimming, returning to gelatinous hosts to mate (Harbison et al. 1977; Laval 1980).

1.3.1.2 Parasitic anemones

Anemones of the genus *Peachia* parasitise jellyfish as juveniles and then leave their host and become typical, free-living, benthic anemones. *Peachia quinquecapitata* is believed to infect the hydromedusan host *Clytia gregaria* by being ingested by the jellyfish as planulae larvae (Spaulding 1972). The anemones remain in the gastrovascular cavity of the host until they are sufficiently developed to commence an ectoparasitic lifestyle. They then move to the subumbrella of the host and feed on the gonads where they mature. After an

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average of 31 days they release from their host and sink to the benthos where they commence a free-living burrowing existence. Adults release planulae to complete the cycle (Spaulding 1972). The anemone *Peachia hillii*, which parasitises the scyphozoan *Catostylus mosaicus* in eastern Australia, has a similar life cycle.

1.3.2 Multiple host life cycles

1.3.2.1 Digeneans

The life cycles of digeneans are usually complex with at least two hosts - one or more intermediate hosts and a definitive (final) host (Cribb 2005). Generally the first intermediate host of a digenean is a mollusc (with the exception of one family which may infect polychaetes) and the definitive host is a vertebrate. A large proportion of digeneans also have a second intermediate host, which include invertebrates and vertebrates. Jellyfish act as second intermediate (e.g. Køie 1975), or paratenic (Køie 1991) hosts. In some (rare) cases an invertebrate is the definitive host (Tripp and Turner 1978). An example of the life cycle of a digenean that parasitises jellyfish is given below (adapted from Cribb 2005 unless otherwise indicated). Sexual adults (Figure 1-1) in a definitive fish host produce eggs that pass to the environment, most likely in the fish's faeces. The eggs hatch to release a motile, ciliated larva, the miracidium. The miracidium usually swims and infects a molluscan first intermediate host where it develops into a mother sporocyst, which reproduces asexually to produce a second intramolluscan generation. This generation may be formed of multiple daughter sporocysts or multiple rediae. These then asexually reproduce themselves by producing daughter sporocysts, rediae or cercariae. The morphology of the cercariae varies greatly but they almost always have a tail. The cercariae emerge from their mollusc host, often ejected through respiratory currents, and may then disperse by water currents or actively swim, using their tail. The cercaria then infects a second intermediate jellyfish host where it develops into a metacercaria. The metacercaria is an important resting stage that allows the parasite to survive until it reaches the definitive host (Martorelli 2001). When the jellyfish is eaten by a definitive host (e.g. *Scomber scombrus*, Køie 1975) it develops into a sexual adult.

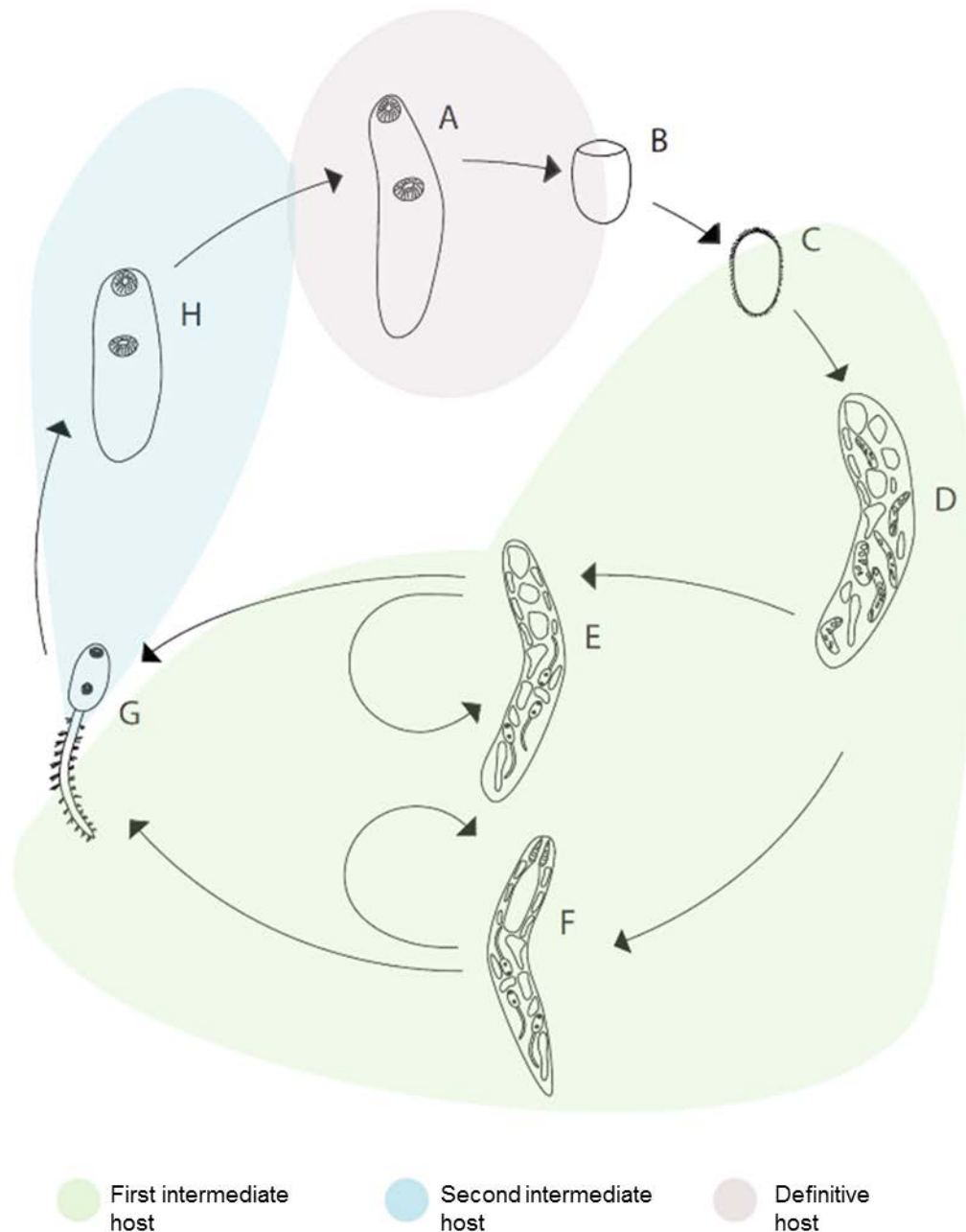


Figure 1-1 Diagrammatic representation of a digenean three host life cycle. A sexual adult (A) produces an egg (B) which hatches into a miracidium (C). This enters the first intermediate host where it develops into a mother sporocyst (D) which produces either daughter sporocysts (E) or rediae (F). These may reproduce themselves or release cercariae (G). A cercaria leaves the mollusc and infects a second intermediate host in which it develops into a metacercaria (H). When this host is eaten by a definitive host, the metacercaria matures into a sexual adult (A)

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1.3.2.2 Cestodes

The life cycle of marine cestodes are poorly known (Beveridge 2001), with complete life cycles determined for only a few species (Caira and Reyda 2005). Similarly to digeneans, most marine cestodes utilise as least one intermediate host and vertebrates act as definitive hosts. However, whilst digeneans often have free living stages in the life cycle, cestodes generally rely solely on trophic interactions to move between hosts. No complete life cycle of a jellyfish-infecting cestode has been determined, however, several cestode larvae that infect ctenophores (Lauckner 1980) and medusae (Southwell 1930; Phillips and Levin 1973) have been identified as belonging to the group 'Tetraphyllidea'. This is a paraphyletic group (Caira et al. 2014), however, many share a 'typical' life cycle. This involves an egg being consumed by a copepod, euphausiid or mollusc. They then develop into a plerocercoid in an invertebrate or fish host (or a merocercoid, commonly found in cetaceans) and may also pass through one or more paratenic hosts. Once consumed by an appropriate elasmobranch (sharks and/or ray) or rattfish (order Holocephali), they transform into the adult stage (Caira and Reyda 2005).

1.4 Mode of transmission

Transmission of parasites from one host to another may be passive, where an infective stage is ingested by the next host in the life cycle, or may be active whereby the parasite directly penetrates the subsequent host (Marcogliese 2004). The parasitic sea anemone *Edwardsiella lineata* is passively transferred from its ctenophore host *Mnemiopsis leidyi* to the ctenophore *Beroe ovata* when *B. ovata* consumes *M. leidyi* (Reitzel et al. 2007). In another example of passive transmission, jellyfish infected with metacercariae of the digenean *Opechona pyriforme* were eaten by the fish *Stenotomus chrysops* under experimental conditions. After dissection four weeks later, mature adults of the digenean were found in the fish (Stunkard 1969). In nature, definitive hosts of *O. pyriforme* include the medusivorous (Bigelow and Schroeder 1974) fish *Hyperoglyphe perciformis* (Linton 1900). Therefore, the digenean is probably transmitted from jellyfish to fish hosts through predation in the wild. Predators of jellyfish include other jellyfish (review: Purcell 1991; Arai 2005b), nudibranchs (e.g. Brandon and Cutress 1985), cephalopods (e.g. Heeger et al. 1992), crustaceans (e.g. copepods, cladocerans, euphausiids, gammaridean amphipods, ostracods and mysids, Hopkins 1985), pycnogonids (e.g. Martin and Kuck 1991), at least 124 species of fish (Arai 1988; Purcell and Arai 2001; Arai 2005a; Pauly et al. 2009), turtles (e.g. Heithaus et al. 2002) and marine birds (review: Arai 2005b). Hence, parasites in jellyfish may be transmitted to many potential hosts via predation.

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Jellyfish may obtain parasites by consuming other hosts. They consume a wide variety of prey from phyto- and zooplankton to small fish (e.g. Zeldis et al. 1995; Carrette et al. 2002). The hemiurid digenean *Lecithocladium excisum* infects copepods in experimental conditions, and occurs in the ctenophore *Pleurobrachia pileus* in the wild. Presumably the ctenophores were parasitised by ingesting infected copepods (Køie 1991). Jellyfish can be voracious predators with high clearance rates (e.g. Purcell et al. 1994). In Port Phillip Bay, the scyphomedusa *Cyanea capillata* (a likely misidentification, it is more likely to be *Cyanea annaskala*, see Dawson 2005a) has been estimated to remove 19.6% of flounder eggs and yolk-sac larvae over their development period (Fancett and Jenkins 1988). Many zooplankton species (e.g. copepods chaetognaths, euphausiids) act as parasite hosts (Marcogliese 1995) and could be sources of parasites in jellyfish.

Jellyfish may also be infected by parasites after feeding directly upon them. The hydromedusa *Clytia gregaria* is infected by the anemone *Peachia quinquecapitata* after feeding upon the planula larvae (Spaulding 1972). The planulae do not actively seek out hosts, but are positively phototactic and swim towards the surface where they are available to be consumed by jellyfish. They can survive for up to 30 days without a host enhancing their chances of being eaten by a suitable host (Spaulding 1972).

Transmission of parasites to jellyfish may also occur through active penetration. For example, the cercariae of the digenean trematode *Opechona bacillaris* emerge from their snail hosts into the water column and penetrate the ctenophore *Pleurobrachia pileus* and the hydromedusa *Eutonia indicans* (Køie 1975). Parasites may also actively transfer between jellyfish hosts. Juveniles of the anemone *Peachia quinquecapitata* can adhere to a new jellyfish host while still attached to another, and then relocate onto the new host as it swims away (Spaulding 1972). Some species of female hyperiid amphipods are thought to swim between hosts whilst depositing their larvae (Laval 1980; Dittrich 1987). Such behaviour would increase the chances of the young surviving, as well as avoiding overpopulating hosts (Laval, 1980).

1.5 Ecology of parasite / host associations

1.5.1 Prevalence and intensity

Prevalence and intensity are useful measures for conveying the spread and importance of a parasite to its host population, species or community. They are widely used indicators, especially since specific definitions were published by Margolis et al. (1982) and Bush et al. (1997). Prevalence is defined as “the number of hosts infected with one or more

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individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species” (Bush et al. 1997). Intensity is “the number of individuals of a particular parasite species in a single infected host” (Bush et al. 1997). Intensity is an important measure because often the heavily parasitised hosts incur higher mortality rates (Poulin 2000).

Hyperiid amphipods can be highly prevalent in jellyfish populations and at times infect 100% of a sampled population (Towanda and Thuesen 2006). The intensity of infection can also be high and individual jellyfish may host hundreds of hyperiids (Dittrich 1988; Towanda and Thuesen 2006). Temporal studies of jellyfish populations show that prevalence and intensities of infection change through time and are linked to the life cycle of the parasites and the host jellyfish. The ability of hyperiid amphipods to deposit many eggs at a time directly onto jellyfish hosts means they are able to infect many medusae with many juveniles, which allows populations of amphipods to increase rapidly. Large numbers of eggs (up to 456/female amphipod) are produced by *Hyperia galba* and intensities of infection may attain 486 amphipods per jellyfish (Dittrich 1988). In just three months, the prevalence of infection by *H. galba* may increase from 0 to almost 100% of the jellyfish population. The rapid population rise of the amphipods is assisted by the presence of mature gonads within jellyfish hosts, which provide a rich food for the amphipods (Dittrich 1988).

The anemone *Peachia quinquecapitata* may also rapidly attain high prevalences in jellyfish populations. For example, prevalences increased from 0 to 65% in less than two months in *Clytia gregaria* (Spaulding 1972). Early studies of *Peachia* revealed low average intensities of approximately three to 10 (Dendy 1889; McDermott et al. 1982), however, a recent study by Riascos et al. (2013) found high intensities with a mean intensity of 465 anemone per host jellyfish.

Prevalence of digeneans in jellyfish is often higher than in other zooplankton (Marcogliese 1995). Copepods, for example, commonly have prevalences of 0.01% to 1% (Marcogliese 1995). In studies where at least 1400 individuals of each jellyfish species were examined for individual digenean species, prevalences of infection ranged from 0.1 to 97.6% (Yip 1984; Girola et al. 1992; Martorelli 1996; Gómez del Prado-Rosas et al. 2000; Diaz Briz et al. 2012). These prevalences varied according to digenean species, host species and season. Reasons for the disparity in prevalence between jellyfish and other planktonic hosts may include: (1) because jellyfish can act as paratenic hosts for some digenean species (e.g. Køie 1991) and because many have high zooplankton clearance rates (e.g.

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Olesen 1995), they may consume large numbers of infected zooplankton and accumulate many metacercariae; (2) jellyfish also may be penetrated directly by species for which they act as paratenic hosts, as well as those for which they are second intermediate hosts (possibly simultaneously as different species can co-exist in one host, e.g. Yip, 1984); (3) the sheer abundance of small crustaceans (e.g. copepods) may be exponentially greater than that of cercariae in surrounding waters; and (4) species able to penetrate jellyfish may be more successful at doing so, than those that penetrate copepods. The relatively few studies of digeneans in all zooplankton (Marcogliese 1995) makes comparison and explanation difficult.

Intensity of digeneans in jellyfish is often high. Maximum intensities have reached 140 in natural infections of a ctenophore for one digenean species (Fraser 1970) and 352 of three digenean species combined in the scyphomedusae, *Aurelia* sp. (Ohtsuka et al. 2010). The larger size and longer life span of jellyfish relative to other zooplankton allows accumulation of a greater number of parasites over time. In addition, the release of mucus by jellyfish may increase their attraction to parasites, as has been shown in fish (Kearn 1967).

1.5.2 Temporal variation in host/parasite associations

Examining variation in infection parameters over time is important for understanding the population dynamics of the parasite and also how parasite loads may affect hosts. Few studies, however, have examined temporal variability in jellyfish/parasite associations. Yip (1984) reported seasonal variation in infection of the ctenophore *Pleurobrachia pileus* with *Opechona bacillaris* and didymozoid larvae in Galway Bay, Ireland. Maximum prevalences occurred in early summer and there were no or few infected jellyfish during winter. Fraser (1970) found that *O. bacillaris* (identified as *O. retractilis* which has since been synonymised) parasitising *Pleurobrachia pileus* in the northern North Sea and north-west areas of Scotland, had much greater intensities in winter than the rest of the year. Ctenophores were sampled monthly for 3 years and over 3000 ctenophores were examined for digeneans. There was a sudden increase in *O. bacillaris* between August and September with abundance increasing from 17-35 metacercariae per 1000 *P. pileus* between March and August to 450-4720 metacercariae per 1000 *P. pileus* in the colder months of September to February. Intensity was particularly high in September with 140 *O. bacillaris* individuals found in one ctenophore which measured only 12 mm. Fraser (1970) concluded that the sudden increase in September was due to an increase in infection at this time, rather than a cumulative increase with host age. Yip (1984)

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concluded that the seasonal difference in maximum infection between her study and that of Fraser (1970) was due to the timing of maximum abundance of the ctenophores which differs in the two locations.

1.5.3 *Spatial variation in host/parasite associations*

There have been few studies of spatial variation in jellyfish parasite relationships, as most studies have been conducted in just one location. Although some jellyfish parasites have wide distributions, they may have different hosts in different areas. The amphipod *Hyperia galba* is found in the seas of the Polar Basin, the North Atlantic and the North Pacific. In the German Bight in the North Sea it parasitises the scyphomedusae *Aurelia aurita*, *Chrysaora hysoscella*, *Rhizostoma pulmo*, *Cyanea capillata* and *Cyanea lamarckii* (Dittrich 1988); in Strangford Lough in Northern Ireland it also parasitises *A. aurita*, *C. lamarckii* and *C. capillata*; in the north American waters of the Atlantic it is similarly found on *C. capillata* and *A. aurita* but additionally parasitises *Tima formosa* (Bowman et al. 1963); whilst in the northern Pacific waters of Japan, it is found on *Chrysaora melanaster* and *Aurelia limbata* (Ohtsuka et al. 2010). With the different hosts, different methods, and different measurements, as well as temporal differences, it is difficult to compare the results.

Two studies have compared the spatial variation of digeneans in jellyfish hosts in the south-western Atlantic. The studies were undertaken over different spatial scales and examined different numbers of digeneans and host species. The digenean *Monascus filiformis* infected four species of jellyfish in three zones off the Argentinian/Uruguayan coasts, covering (Girola et al. 1992). Prevalence of infection was lowest in *Phialidium* sp., and was also low in *Liriope tetraphylla* in the zone nearest the mouth of the Río de la Plata. The authors concluded that the freshwater input of the river affected the patterns of spatial distribution of digeneans in the jellyfish. In a larger scale study of *Monascus filiformis* and three other digenean species over the Brazilian, Uruguayan and Argentinean continental shelf, areas with high prevalences of infection correlated with oceanographic fronts (Diaz Briz et al. 2012). Frontal areas are often regions of intense trophic activity (Graham et al. 2001) that may well promote transmission of parasites which rely on predation.

1.6 How technical advances have improved understanding of jellyfish / parasite associations.

The majority of early studies of jellyfish parasites were descriptive and focused on taxonomy (Harbison et al. 1977; Browne unpubl.). In the case of ectoparasites, associations were often overlooked because sampling with towed nets easily separates ectoparasites from their hosts (Harbison et al. 1977). Difficulties in identifying larval stages (e.g. of digeneans and cestodes) have also hampered the study of jellyfish parasites. Even identifications of adult parasites can be problematic and parasites may be misidentified (e.g. White and Bone (1972) misidentified *Hyperia macrocephala* as *Hyperia galba*). It is only in more recent years that methods have developed to allow careful sampling and recording of associations (e.g. through SCUBA, blue water diving and video techniques) and sampling has been done over spatial and temporal scales, measuring a variety of factors, such as intensity, prevalence and host size. There have also been several large studies at population levels (Yip 1984; Mills 1993; Diaz Briz et al. 2012). Even with these more recent changes in technology, the parasites of jellyfish are still not well understood.

The determination of life cycles of parasites, particularly those with multiple hosts, is difficult due to the different morphologies of each life stage and the challenge of tracing a parasite through its different hosts. The taxonomy of digeneans is based on the reproductive organs of the adult stage and the absence of sexual organs in early life stages means it can be difficult to link the appropriate life history stages to each other. In the past, the life cycles of jellyfish digeneans were elucidated using “feeding experiments” (e.g. Køie 1975; Stunkard 1978). These experiments were undertaken in a laboratory and usually involved observing cercariae emerge from a mollusc host and penetrate a jellyfish host. Infected jellyfish hosts (or metacercariae implanted into mussel or similar tissue) were then fed to fish hosts, which often were not the natural definitive host. Several weeks later the fish were dissected and (ideally) adult trematodes obtained. Such experiments were often combined with sampling naturally infected wild hosts (e.g. Køie 1985). As there are only 11 species of digeneans which infect jellyfish for which complete life cycles are known (Browne unpubl.), there may be alternative infection strategies which have not yet been discovered (as well as more species which use jellyfish as hosts). DNA technology can now be used to match sequences of unidentified larval forms, to sequences from known species of adults (Adlard et al. 1993; Nolan and Cribb 2005). Molecular approaches can also be used to differentiate between morphologically similar species or investigate relationships within species complexes (Cribb et al. 1994). The use of DNA technology

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creates exciting opportunities for investigating life cycles without the need for time and labour-intensive feeding experiments and keeping animals in captivity for long lengths of time (Cribb et al. 1998).

1.7 Thesis Objectives

The main objectives of this thesis are to increase understanding about jellyfish parasites and their interactions with hosts in the marine environment. Specific aims include:

- to use morphology and molecular techniques to identify digenean metacercariae from jellyfish, by comparing ITS2 sequences and morphology of the metacercariae with those of adult digeneans in fish;
- to suggest other hosts and potential parts of the life cycle of the digenean parasites of jellyfish using morphological and molecular techniques;
- to compare spatial and temporal variation in digenean metacercariae in jellyfish, using variations in morphology and molecular biology to distinguish between species;
- to determine and compare prevalences, intensities of infection and host specificity of digenean metacercariae in a range of jellyfish hosts; and
- to investigate the parasites of the scyphomedusa *Catostylus mosaicus* over time, and measure biological characteristics to elucidate the life cycles of the parasites and the relationship between the parasites and the jellyfish.

1.8 Thesis Structure

Chapter One provides a general background of jellyfish parasite research, outlining the relevance of jellyfish parasites and the limited information available. In Chapter Two, two common digeneans which infect jellyfish in Port Phillip Bay are investigated. The metacercariae of the two digenean species (hosted by three species of jellyfish) are identified and described, using molecular and morphological techniques. Other hosts and potential part life cycles for the digeneans are proposed and discussed. In Chapter Three, the endoparasites of one jellyfish species are compared between different locations to determine whether spatial variation occurs. Digenean parasites of *Cassiopea* sp. are compared between four different locations using morphological and molecular techniques and a species of digenean from *Cassiopea* sp. is identified and described. In Chapter Four, the biodiversity of digenean fauna of jellyfish from one area, Lizard Island, is investigated, to determine whether different jellyfish species host similar digeneans. Digeneans obtained from five different jellyfish species are differentiated using morphology

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and DNA sequencing. Differences in intensity, prevalence and host specificity of the digeneans were measured and compared. In Chapter Five, the parasites of one jellyfish species are explored over time, to determine whether temporal variation occurs. Temporal patterns in the intensities and prevalences of parasites of *Catostylus mosaicus* over two years are investigated. Information gathered from the parasites is used to explain the life cycles of the parasites and the relationship between the jellyfish and its parasites. In Chapter Six the overall findings of the thesis are synthesised and the relevance of the findings discussed.

2 Molecular identification of lepopocreadiid trematode parasites infecting jellyfish in Port Phillip Bay, Australia

2.1 Introduction

Jellyfish act as important intermediate hosts for digenean trematodes (e.g. Køie 1975; Martorelli and Cremona 1998; Diaz Briz et al. 2012); however, research on this relationship is hampered by the difficulty of confidently identifying the trematode species in their jellyfish hosts. Digenean identification is based upon reproductive organs in the adult form, many of which are lacking in the metacercaria stage found in second intermediate hosts (such as jellyfish). Analysis of DNA allows the differentiation of digenean species using the ribosomal DNA spacer ITS2 (Anderson and Barker 1993; Nolan and Cribb 2005). Sequences from unidentified metacercariae can be matched to those of known adults. Coupled with morphological data and knowledge of host biology, insights can be made into the life cycles of the parasites.

Jellyfish act as intermediate hosts for at least 17 digenean species, and digeneans are hosted by approximately 68 species of jellyfish (medusae, siphonophores and ctenophores) (Browne unpubl.). In the past, life cycles of digeneans were primarily determined using feeding experiments (e.g. Stunkard 1980b). Lepocreadiid cercariae that infected jellyfish in these studies left their first intermediate mollusc host and swam in the water column until directly penetrating the second intermediate jellyfish host and forming metacercariae (Stunkard 1969; Stunkard 1972; 1980a; 1980b; Køie 1975). When the jellyfish were eaten by fish, the metacercariae developed into sexual adult digeneans (Stunkard 1969; 1980a; 1980b). Predation by fish upon jellyfish has probably been underestimated because of their low caloric value (Arai 1988; Bullard and Hay 2002), unpalatability (Bullard and Hay 2002), fast digestion rates (Arai et al. 2003) and the difficulty of identifying jellyfish in gut contents, particularly after fish have been preserved or frozen (Arai 1988; Arai et al. 2003). However, at least 124 species of fish prey upon jellyfish (Arai 1988; Purcell and Arai 2001; Arai 2005a; Pauly et al. 2009). As digeneans in jellyfish are often more prevalent than in other zooplankton intermediate hosts such as copepods (Marcogliese 1995), jellyfish may be more important as hosts than previously believed. The use of molecular identification may reveal more digenean species that use jellyfish as hosts.

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This study utilised the internal transcribed spacer 2 (ITS2) of ribosomal DNA. The ITS2 region is highly variable yet relatively conserved within platyhelminth species, and thus is useful for distinguishing between species and hence for identifying larval forms (Adlard et al. 1993; Tandon et al. 2007; Skov 2009). The aims of this study were to use molecular techniques to identify metacercariae from jellyfish, by comparing ITS2 sequences of the metacercariae to those of adult digeneans in fish that associate with jellyfish, and to investigate the role of jellyfish in the life cycles of digenean parasites.

2.2 Methods

2.2.1 Host species

Three jellyfish host species were sampled. All species were transparent, enabling digeneans to be easily and quickly located with the aid of a dissecting microscope. The lion's mane jellyfish, *Cyanea annaskala* is a scyphozoan (order Semaestomeae) found in cold temperate waters of south-eastern Australia (Dawson 2005a). It has a lobed bell and numerous fine tentacles (Figure 2-1a). Haeckel's jellyfish, *Pseudorhiza haeckeli*, is also a scyphozoan (order Rhizostomeae). It is more widely distributed, found in south-western and southern Australian and the Northern Territory (Southcott 1982). It has a distinctive paddle-shaped appendage attached to its oral arms (Figure 2-1b). It is a mostly oceanic species (Southcott 1982). The hydromedusa *Eirene menoni* and juvenile white trevally *Pseudocaranx dentex* may shelter under the bell of this medusa (Southcott 1982). *Aequorea eurodina* is a hydrozoan jellyfish (order Leptothecata) with a lens shaped bell, and numerous tentacles (Figure 2-1c). The original description of this species is poor and its taxonomic relationship with the cosmopolitan species *Aequorea forskalea* requires resolution (Edgar 1997).

Three species of fish were sampled: the blue warehou, *Seriolella brama* (Figure 2-1d and e); the silver warehou, *Seriolella punctata* (Figure 2-1f); and the blue mackerel, *Scomber australasicus* (Figure 2-1g). The warehou occur in south-eastern Australian and New Zealand waters (Gomon et al. 2008) and are commercially important (Bruce et al. 2001). Juveniles of *S. brama* have been recorded in bays and estuaries of Tasmania, sheltering under the scyphozoan identified as *Cyanea capillata* (likely to be *C. annaskala*, see Dawson 2005), while *S. punctata* juveniles live offshore under jellyfish (Last et al. 1983). Adult *S. brama* and *S. punctata* live on the continental slope and undertake spawning migrations in winter or winter-spring in south-eastern Australia (Bruce et al. 2001). The mackerel *S. australasicus* has a much broader distribution, occurring in the western Pacific from Australia and New Zealand north to Japan, east to Hawaii and

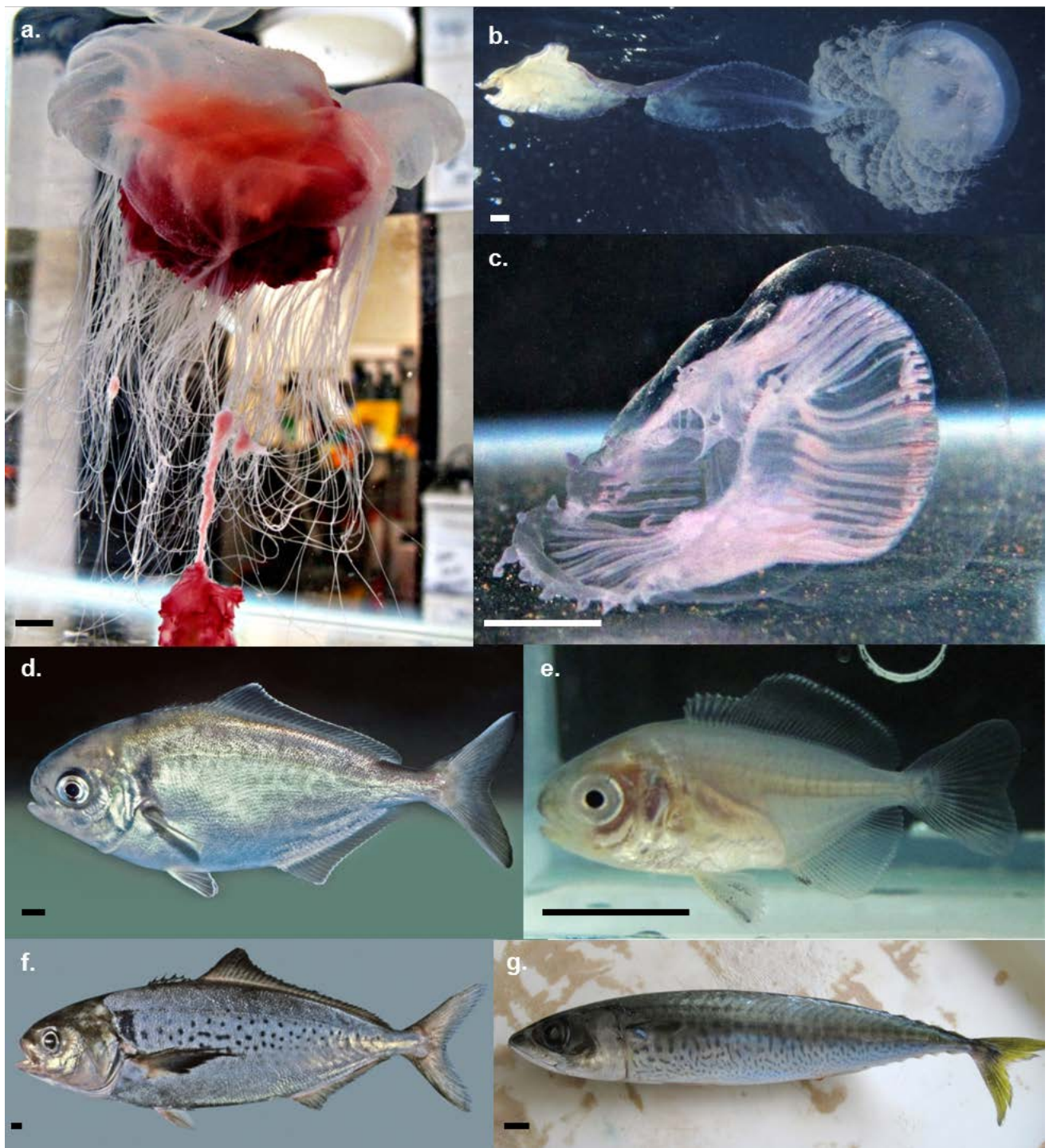


Figure 2-1 Study animals (a) *Cyanea annaskala* (b) *Pseudorhiza haeckeli* (c) *Aequorea eurodina* (d) *Seriolella brama* (adult) (e) *Seriolella brama* (juvenile) (f) *Seriolella punctata* (g) *Scomber australasicus*. Scale bars represent 1 cm. Image (b) *Pseudorhiza haeckeli* by [Mark Norman](#) / Museum Victoria is licensed under [CC by 3.0](#). Images (d) *Seriolella brama* and (f) *Seriolella punctata* reproduced with permission from [Rudie H. Kuiter](#)/Aquatic Photographics © all rights reserved

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Socorro Island of Mexico, the Red Sea and the Persian Gulf (Collette 2003). It is distributed around the entire coastline of Australia, except for north-west Western Australia (Gomon et al. 2008). *Scomber australasicus* is a small, shoaling fish found in coastal waters and open sea (Gomon et al. 2008) with a peak spawning season in southern Australia from December to March (Rogers et al. 2009). Three species within the genus *Scomber* are parasitised by digeneans (*Opechona bacillaris* and *Lecithocladium excisum*) which are known to use jellyfish as hosts (Gibson and Bray 1986; Bray and Gibson 1990; K  ie 1991; Costa et al. 2011).

2.2.2 Collection of specimens

Jellyfish were sampled opportunistically from six locations within Port Phillip Bay (38°05'17"- 38°17'1"S, 144°36'54"-144°43'58"E) between September 2009 and February 2012 (Figure 2-2). Port Phillip Bay is a large, shallow embayment with a surface area of approximately 1930 km². It is separated from Bass Strait by an entrance approximately 3 km wide. Three *Cyanea annaskala* individuals, seven *Pseudorhiza haeckeli* and six *Aequorea eurodina* were collected. Jellyfish were collected with a dip net from a boat, or by zip lock bag whilst snorkelling or after being washed ashore. Those that had been washed ashore (only *A. eurodina*) had begun to disintegrate, however, medusae were still identifiable and the digenean metacercariae within the medusae were still alive. Any other organisms associating with the jellyfish were recorded. Jellyfish were returned to the laboratory (alive where possible) where bell diameter was measured. They were examined for parasites under a Leica Wild M8 stereomicroscope using transmitted light.

Three fish species known to feed upon jellyfish were obtained between June 2009 and April 2011 from a provider of bait for recreational fishers (*Scomber australasicus*), a Victorian Department of Primary Industries trawl survey (*Seriolella brama*) and a commercial fisher (*Seriolella punctata*). Five *S. brama* were trawled within Port Phillip Bay, Victoria (37°58'30"-38°14'34"S, 144°46'01"-144°52'38"E, three *S. punctata* trawled off Portland, Victoria (estimated within the vicinity of 38°20' S, 141°36'E) and two *S. australasicus* individuals were caught in Moreton Bay, Queensland (27°07'54.28" S, 153°21'10.63" E). *Scomber australasicus* were sampled in relation to research on tropical jellyfish species, hence the large distance from the other collection sites. However, due to the wide distributions and overlapping ranges of *S. australasicus* and *Aequorea* species, this widely-dispersed sampling was considered worthy of investigation.

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The sampled fish were dead for 10-48 hours prior to being dissected. During this time they were refrigerated or stored on ice (*S. punctata* and *S. brama*) or frozen (*S. australasicus*). In the laboratory, the total length of each fish was measured, and the digestive tract was removed and separated into stomach, pyloric caeca, and intestine. Each section was examined for digeneans using a stereomicroscope, then shaken vigorously in a jar of vertebrate saline (1 part seawater: 3 parts freshwater) before being re-examined (following the technique of Cribb and Bray, 2010). The solution was then allowed to settle for 1-2 minutes and three-quarters of the supernatant was discarded and the remaining fluid was examined under the microscope (Cribb and Bray 2010).

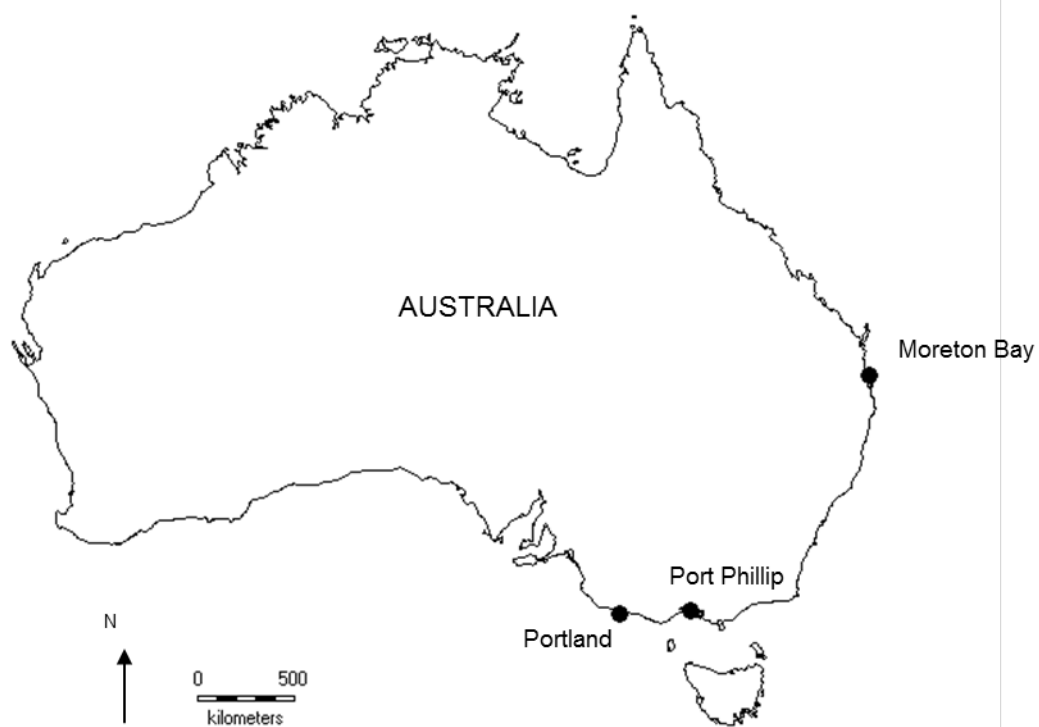


Figure 2-2 Sampling locations Moreton Bay (QLD), Port Phillip and Portland (VIC)

2.2.3 Treatment of digeneans

The location of the digeneans within the fish or jellyfish was recorded and they were assigned to a morphotype. The digeneans were removed from the jellyfish or fish/gut washes with a pipette and immersed into near-boiling vertebrate saline. This method (Cribb and Bray 2010) straightens the digeneans uniformly, thereby allowing individuals and species to be compared. They were then fixed in 10% formalin for morphological analysis or 96% absolute alcohol for molecular sequencing. The number of digeneans of

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each morphotype per jellyfish was recorded. Prevalence and mean intensity (see Glossary) of each morphotype were calculated according to Bush et al. (1997).

2.2.4 *Morphological analysis*

Prior to DNA extraction, ethanol-preserved samples were viewed in fresh water on a concave slide using an Olympus BX50 compound microscope. Photographs were taken with a QImaging Go-21 CMOS camera mounted on the microscope and measurements were made using an ocular micrometer. The following morphological characters were measured: body length, body width, length of forebody, oral sucker width, oral sucker length, ventral sucker width and ventral sucker length. Forebody refers to the distance between the anterior extremity of the body and the posterior margin of the ventral sucker. The distance to pharynx was measured from distal end of oral sucker to proximal end of pharynx.

The digeneans preserved in formalin were rinsed in water and over stained with Mayer's haematoxylin. They were then rinsed in fresh water, destained with 1% HCl acid then neutralized in 1% ammonium hydroxide solution (Miller and Cribb 2007). The specimens were dehydrated through a series of ethanol solutions between 70 and 100%, cleared in methyl salicylate and mounted in Canada balsam. The digeneans were viewed using an Olympus BX50 compound microscope and drawn with a camera lucida. Images were digitized using a Wacom tablet and Adobe Illustrator. Measurements were made using an ocular micrometer. Measurements are given in Table 2-3 for each host and preservative. In species descriptions (Sections 2.3.2.1-2.3.2.1) some proportional measurements for each digenean species are given in the species description as the range with the mean in parentheses.

2.2.5 *Molecular analysis*

Genomic DNA was isolated from single specimens using proteinase K and either the phenol:chloroform extraction procedure (Sambrook et al. 2001) or a QIAamp® DNA Mini Kit. Due to the small size of the trematodes (some <200 µm), the following modifications were made. Prior to extraction, each trematode was pipetted into a vial with a minimal amount of ethanol. The vial lids were left open until the ethanol had evaporated (removing the risk of losing the digenean when aspirating off solution). TE buffer and proteinase K were added, the solution was centrifuged and vortexed and then placed overnight in a rotating incubator. Amplification of the ITS2 region was performed using the forward primer "3S" (5'-GGTACCGGTGGATCACGTGGCTAGTG-3) (3' end of 5.8S rDNA) (Bowles et al.

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1993) and the reverse primer “ITS2.2” (5'-CCTGGTTAGTTTCTTTTCCTCCGC-3') (5' end of 28S rDNA) (Cribb et al. 1998). The PCR reactions were carried out in 20 µl volumes, each with 4 µl of Hotstar Q solution (QIAGEN), 2 µl of 10x PCR reaction buffer, 0.8 µl of 10 mM dNTP, 0.75 µl of each primer (Invitrogen) (10 µM), 0.25 µl of HotstarTaq (QIAGEN), 6.45 µl of nuclease-free H₂O, and 5 µl of template DNA. Amplifications were undertaken using the following protocol: an initial step of 95°C for 15 min followed by 35 cycles of denaturation at 96 °C for 45 s, annealing at 48 °C for 30 s and extension at 72 °C for 45 s followed by a final extension step of 72 °C for 4 min and a holding temperature of 15 °C. Reamplification was necessary for the metacercariae samples (most likely due to the small size of each specimen). For these reactions 1 µl of PCR product was used instead of 5 µl of DNA template, and 10.45 µl of nuclease free water. The amplification procedure used was the same. Positive and negative controls were run for all amplifications. Even with reamplification the success rate of obtaining sequences of digeneans from *Aequorea eurodina*, and those already dead prior to extraction from *Scomber australasicus* and *Seriolella punctata* was low. The number of each sequences obtained for the digenean species from each host was *Cyanea annaskala* (n=3), *Pseudorhiza haeckeli* (n=4), *A. eurodina* (n=1), *Seriolella brama* (n=4), *S. punctata* (n=1) and *Scomber australasicus* (n=2). The amplified DNA was purified using ExoSAP-IT (GE Healthcare) following the manufacturer's recommended protocol. The purified product was then sent to a commercial company (Macrogen, South Korea) for sequencing.

2.2.6 Phylogenetic analysis

The resulting forward and reverse sequences were edited to produce a single sequence for each specimen using BioEdit version 7.0.9.0 (Hall 1999). As the sequences obtained included the entire ITS2 region and sections of the adjoining 5.8S and 28S, the ITS2 sequence was isolated using the annotation tool of the ITS2 database (<http://its2.bioapps.biozentrum.uni-wuerzburg.de/>) using the default parameters (Keller et al. 2009). Sequences were aligned in MEGA 5.05 (Tamura et al. 2011) using MUSCLE with the defaults selected except maximum iterations which were changed to 10. Alignment was checked by eye in Mesquite (Maddison and Maddison 2011) and the ends were trimmed to match the shortest sequence. Distance matrices were constructed using MEGA 5.05 (Tamura et al. 2011) to calculate the number of base differences per sequence. Pairwise deletion was selected to remove ambiguous positions.

2.3 Results

2.3.1 Prevalence and intensities of infection

Each jellyfish species had only one morphotype of digenean metacercaria and using the DNA results, photographs, and measurements, the species were identified as the lepocreadiids *Cephalolepidapedon warehou* and *Opechona bacillaris* (Table 2-1). *Opechona bacillaris* occurred only within *Aequorea eurodina* whereas *C. warehou* occurred in both *Pseudorhiza haeckeli* and *Cyanea annaskala*. Within the jellyfish hosts, prevalences ranged from 33-100% and intensities varied from 4 to 96 metacercariae per host (Table 2-1). It should be noted these are only preliminary results, obtained from small sample sizes. Metacercariae were distributed in most parts of the jellyfish.

Cephalolepidapedon warehou occurred in the bell, oral arms and gastric cirri of both jellyfish hosts, and also in the oral disc and stomach wall of *P. haeckeli* (Table 2-2). No formal statistical analysis of location was done due to the small sample sizes, and volumes of jellyfish body structures were not measured. *Opechona bacillaris* occurred in the bell of *A. eurodina*. There were no obvious signs of damage to the jellyfish by the digeneans, however, they were easy to dislodge with a pipette suggesting a possible “softening” of the surrounding tissue.

Cephalolepidapedon warehou occurred in both species of warehou examined, *Seriotelella brama* and *Seriotelella punctata*. All five specimens of *S. brama* were parasitised by this digenean, with a maximum intensity of 25 (Table 2-1). Four of the *S. brama* were relatively large (17.5 to 26 cm) and the other specimen was a small juvenile. The juvenile (3.2 cm) had the lowest intensity with five digeneans. *Cephalolepidapedon warehou* were found in two of the three *S. punctata* examined, with a maximum intensity of five. Mature and immature specimens of *C. warehou* were found in both fish species, as expected. All *C. warehou* were recovered from the intestines of both fish species. *Opechona bacillaris* occurred in the intestines of both specimens of blue mackerel *Scomber australasicus* (Table 2-1). Intensity ranged from four to 11. Mature and immature *O. bacillaris* were present.

In addition to digenean metacercariae, the jellyfish *Pseudorhiza haeckeli* hosted numerous associates including the hyperiid amphipods *Hyperia gaudichaudii* and *Themisto australis*, the parasitic anemone *Peachia hilli*, and the sphaeromatid isopod *Cymodoce gaimardii*. One *Cyanea annaskala* jellyfish had six *P. hilli* attached and one *Aequorea eurodina* jellyfish had two *H. gaudichaudii* attached. A juvenile *Seriotelella* sp. was observed

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Table 2-1 Digenean species, species of hosts dissected, number of hosts (n), prevalence (P) of infection, mean intensity of digeneans and range, size of host (bell diameter (BD) for jellyfish, total length (TL) for fish) and date collected . * mean intensity only calculated from 3 fish specimens)

Digenean species	Host species	n	P (%)	Mean intensity±SE (range)	BD/TL (cm)	Months digeneans found
<i>Cephalolepidapedon warehou</i>	Jellyfish					
	<i>Cyanea annaskala</i>	3	33	6 (6)	1.8-8.0	September
	<i>Pseudorhiza haeckeli</i>	7	71	24.0 ± 18.0 (4-96)	4.0-21.0	May, July, October
	Fish					
	<i>Seriola brama</i>	5	100	13.3 ± 6.0 (5-25)*	3.2-26.0	March, April, June
<i>Opechona bacillaris</i>	<i>Seriola punctata</i>	3	67	3.0 ± 2 (1-5)	43.0-48.0	December
	Jellyfish					
	<i>Aequorea eurodina</i>	6	100	15.3 ± 4.4 (5-30)	2.2-3.5	December, February
	Fish					
	<i>Scomber australasicus</i>	2	100	7.5 ± 3.5 (4-11)	22.0-22.5	October

Table 2-2 Number of digeneans within each location of their jellyfish hosts

Digenean species	Jellyfish host species	Location within jellyfish				
		Bell	Stomach	Oral arms	Oral pillar/ disc	oral Tentacle
<i>Cephalolepidapedon warehou</i>	<i>Cyanea annaskala</i>	2		3		1
	<i>Pseudorhiza haeckeli</i>	43	1	72	9	
<i>Opechona bacillaris</i>	<i>Aequorea eurodina</i>	38				

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swimming around several *A. eurodina* on one occasion but was not captured to enable identification to species

2.3.2 Morphological results

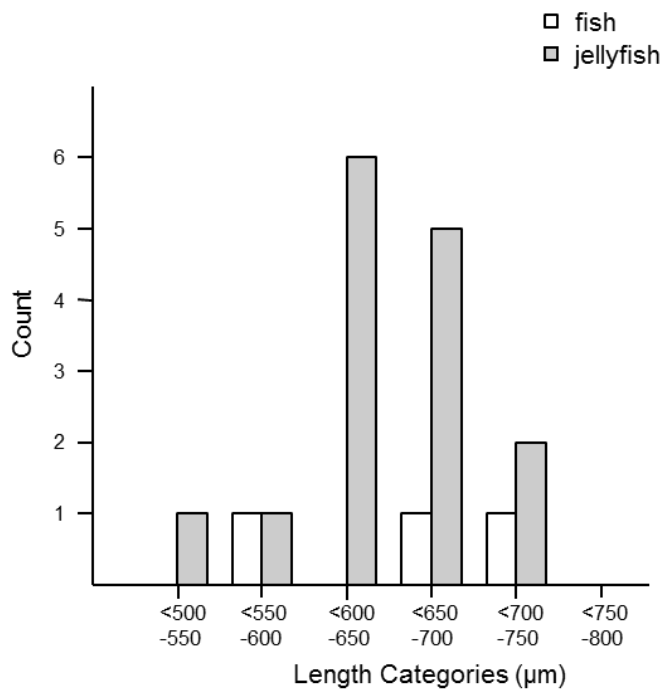
2.3.2.1 Adult *Cephalolepidapedon warehou* from fish hosts

The adult trematodes from *Seriolella punctata* and *Seriolella brama* were identified as *Cephalolepidapedon warehou* (family Lepocreadiidae) using Bray and Cribb (2003). The largest digenean from *S. brama* (790 μm) was larger than the metacercariae from the jellyfish (720 μm) (Figure 2-3, Table 2-3), however, the sizes of measured digeneans from fish and jellyfish hosts were within a similar range. Measurements of *C. warehou* from *S. punctata* are not included in the table as few trematodes were alive when the fish were dissected, and those that were did not straighten appropriately. Attributes of the digeneans from both *warehou* species were consistent with those given for metacercariae below (Section 2.3.2.2). Comparable proportions of *C. warehou* from *S. brama* were as follows: prepharynx long (distance to pharynx 22-31(26) % of body length). Forebody long 39-46% (43) of body length. Sucker-width ratio 1:0.75-1.07 (1:0.91).

2.3.2.2 *Cephalolepidapedon warehou* metacercariae from jellyfish hosts

Measurements in Table 2-3. Body elongate (Figure 2-4a), rounded posteriorly. Tegument spinose, spines in regular rows in forebody, sparse or absent in hindbody. Eye-spot pigment copious, scattered throughout parenchyma of forebody, to about posterior margin of ventral sucker. Oral sucker funnel-shaped, terminal. Ventral sucker slightly smaller than oral, rounded, pre-equatorial on slight protuberance. Prepharynx long (distance to pharynx 22-33 (29) % of body length). Pharynx large, oval. Forebody long 42-53% (47) of body length. Sucker-width ratio 1:0.71-1.15 (1:0.87). Caeca terminate blindly. Testes two, entire, in midhindbody. Rounded to oval.

a.



b.

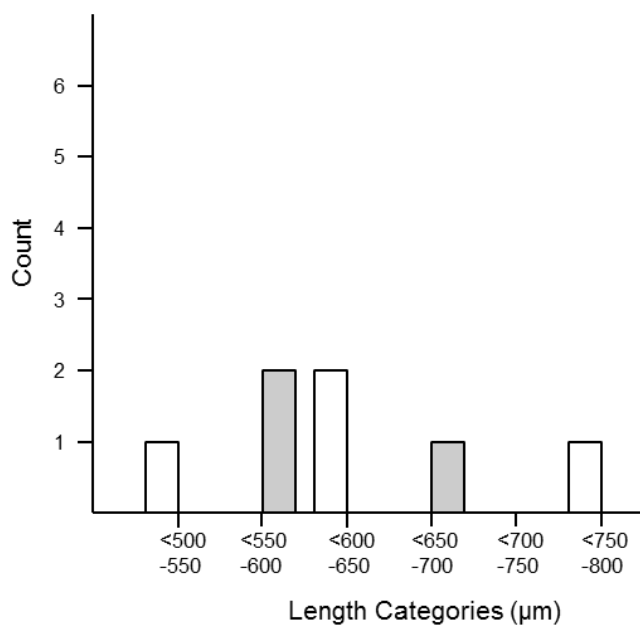


Figure 2-3 Size frequency distribution of *Cephalolepidapedon warehou* length from fish and jellyfish hosts. Lengths of digeneans were pooled from the host jellyfish *Pseudorhiza haeckeli* and *Cyanea annaskala*. The fish host was *Seriotelella brama*. (a) Ethanol preserved samples (b) Slide mounted formalin preserved samples

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Table 2-3 Measurements of digeneans found in jellyfish and fish hosts. Measurements are in micrometres and given as range, mean \pm standard deviation, and number of specimens for which that measurement was obtained. Forebody refers to the distance between the anterior extremity of the body and the posterior margin of the ventral sucker. Sucker width ratio is given with oral sucker as one. Distance to pharynx is measured from distal end of oral sucker to proximal end of pharynx. Body width:oral sucker width ratio is given with body as one

Digenean species Host (preservative)	Length	Width	Forebody length	Oral sucker width	Oral sucker length	Ventral sucker width	Ventral sucker length	Length/ width	Forebody as % of body length	Sucker width ratio	Distance to pharynx as % of body length	Body width: oral sucker width
<i>Cephalolepidapedon warehou</i>												
<i>Pseudorhiza haeckeli</i> (formalin)	580-660	60-80	260-310	70	65-70	60	50-60	7.5-9.67	42.4-53.4	0.857	23.3-32.8	0.875
	613 \pm 41.6	70 \pm 10	283 \pm 25.2	70	68.3 \pm 2.89	60	53.3 \pm 5.77	8.87 \pm 1.19	46.4 \pm 6.12	0.857	27.3 \pm 4.89	0.875
	3	3	3	1	3	1	3	3	3	1	3	1
<i>Pseudorhiza haeckeli</i> (ethanol)	540-710	70-110	250-340	60-80	50-75	50-75	55-70	6.18-9.57	44.4-50	0.714-1.15	27.7-31.3	0.682-1
	635 \pm 47.7	85 \pm 11.7	301 \pm 25.8	70.9 \pm 5.39	62.1 \pm 8.11	61.4 \pm 7.78	61.3 \pm 4.83	7.58 \pm 1.04	47.4 \pm 1.53	0.868 \pm 0.117	29.4 \pm 1.31	0.841 \pm 0.107
	12	12	12	11	12	11	12	12	12	11	10	11
<i>Cyanea annaskala</i> (ethanol)	660-720	90-110	320-340	70-80	60-70	60-70	60-60	6.55-7.78	45.7-51.5	0.75-0.875	26.9-29.4	0.727-0.8
	693 \pm 30.6	100 \pm 10	330 \pm 10	76.7 \pm 5.77	66.7 \pm 5.77	63.3 \pm 5.77	0	6.97 \pm 0.696	47.7 \pm 3.32	0.827 \pm 0.0676	28.1 \pm 1.79	0.768 \pm 0.0373
	3	3	3	3	3	3	3	3	3	3	2	3
<i>Seriolella brama</i> (ethanol)	580-730	80-150	250-330	50-100	55-70	50-80	60-70	4.87-7.33	42.4-45.2	0.75-1	26.2-26.4	0.625- 0.889
	657 \pm 75.1	107 \pm 37.9	287 \pm 40.4	76.7 \pm 25.2	61.7 \pm 7.64	63.3 \pm 15.3	63.3 \pm 5.77	6.48 \pm 1.4	43.6 \pm 1.45	0.85 \pm 0.132	26.3 \pm 0.165	0.727 \pm 0.142
	3	3	3	3	3	3	3	3	3	3	2	3
<i>Seriolella brama</i> (formalin)	490-790	50-120	230-310	70	60-80	75	50-70	5.33-9.8	39.2-46.9	1.07	23.9-30.6	0.583
	638 \pm 123	85 \pm 28.9	275 \pm 34.2	70	72.5 \pm 9.57	75	63.8 \pm 9.46	7.95 \pm 1.91	43.6 \pm 3.35	1.07	27.6 \pm 2.81	0.583
	4	4	4	1	4	1	4	4	4	1	4	1
<i>Opechona bacillaris</i>												
<i>Aequorea</i> sp. (formalin)	500	90	270	50	40	40	45	5.56	54	0.8	ND	0.556
	638 \pm 123	85 \pm 28.9	275 \pm 34.2		72.5 \pm 9.57	75	63.8 \pm 9.46	7.95 \pm 1.91	43.6 \pm 3.35	1.07	27.6 \pm 2.81	0.583
	4	4	4	1	4	1	4	4	4	1	4	1
<i>Aequorea</i> sp. (ethanol)	300-500	58-100	170-270	35-60	35-50	30-50	30-50	4.22-5.67	44.2-56.7	0.75-1	ND	0.5-0.694
	409 \pm 71.8	82.9 \pm 16.3	214 \pm 42.4	47.9 \pm 8.09	39.3 \pm 5.35	41.1 \pm 9.08	41.7 \pm 7.45	4.98 \pm 0.561	52.5 \pm 4.79	0.857 \pm 0.105	ND	0.583 \pm 0.0601
	7	7	7	7	7	7	7	7	7	7	0	7

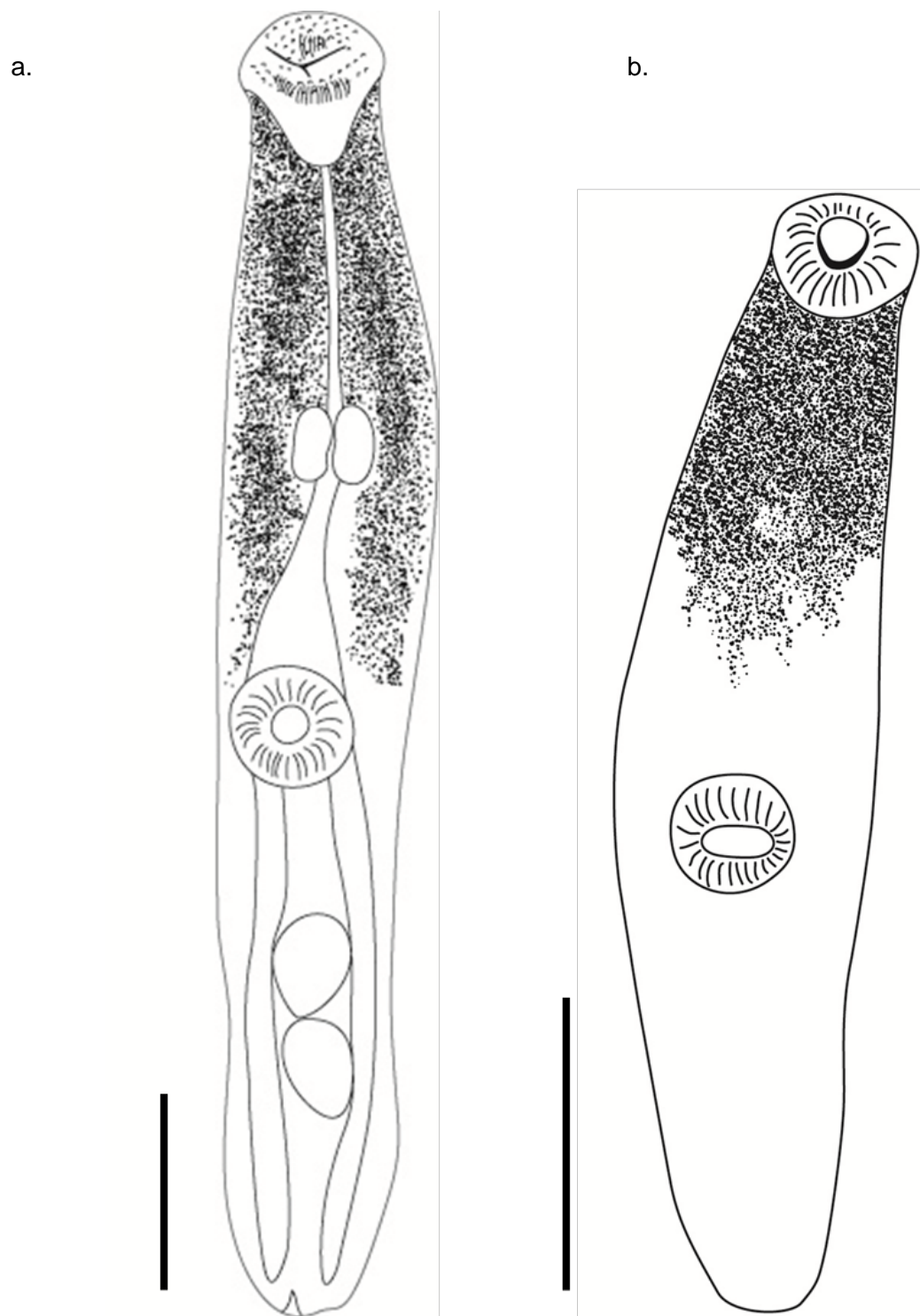


Figure 2-4 (a) Ventral view of *Cephalolepidapedon warehou* metacercaria from *Pseudorhiza haeckeli* from Port Phillip Bay. (The joining of intestinal caeca above ventral sucker not shown as unable to be seen). Drawing a composite of 4 worms. (b) Ventral view of *Opechona bacillaris* metacercaria from *Aequorea eurodina* from Port Phillip Bay. Drawing a composite of 2 worms. Scale bars of (a) and (b) represent 100 μ m

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2.3.2.1 *Adult Opechona bacillaris* from fish hosts

Adult digeneans from *Scomber australasicus* were consistent with the description of *Opechona bacillaris* given by Bray and Gibson (1990). Measurements of adult *O. bacillaris* from *S. australasicus* are not presented as the digeneans were dead and deformed from being frozen when the fish were dissected and so they could not be straightened. Measurements, therefore, are not accurate and unable to be compared with *O. bacillaris* metacercariae from jellyfish. However, morphological characters could still be compared and were similar to those given for metacercariae.

2.3.2.2 *Opechona bacillaris* metacercariae from jellyfish hosts

Opechona bacillaris metacercariae from *Aequorea eurodina* were smaller than those retrieved from the fish. No mature specimens were recovered from jellyfish.

Measurements in Table 2-3. Body elongate (Figure 2-4b). Eye-spot pigment heavy in region from oral sucker to more than halfway to ventral sucker. Oral sucker large, infundibuliform; may be withdrawn into forebody, aperture wide, terminal or slightly ventrally subterminal. (Prepharynx, pharynx, oesophagus and pseudoesophagus obscured by pigment and could not be seen in these samples). Intestinal bifurcation in forebody (exact location unable to be seen due to heavy pigmentation).

Forebody long 44-57% (52), ventral sucker rounded; smaller than oral sucker, sucker-width ratio 1:0.75-1 (0.86), in posterior half of body; slightly protuberant (differs to adult which is in anterior third of body and distinctly smaller than oral sucker). Excretory pore terminal. No testes, seminal vesicle, ovaries, or other sexual organs visible.

2.3.3 *Molecular results*

Sequences of *Cephalolepidapedon warehou* from seven adult digeneans from *Seriolella brama* and *Seriolella punctate* were identical to those from seven metacercariae in *Pseudorhiza haeckeli* and *Cyanea annaskala* (Table 2-4). The ITS2 sequences were annotated to begin with 5'-GCTTATAAAC-3' and end with 5'-GTACTTCATT-3'. All of the sequences contained complete ITS2 sequences of 290 bases, except two shortened sequences from *C. annaskala* metacercariae. These truncated sequences were missing three and 72 bases from the 3' end, most likely due to sequencing error caused by the low concentration of DNA that was obtained from each specimen (the larger adult samples had a higher success rate than metacercariae). Apart from the missing ends of these two sequences, all sequences were identical except one from one specimen from *S. brama*.

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This sequence differed by only one base. Sequences of *Opechona bacillaris* from two adult digeneans in *Scomber australasicus* matched sequences from two metacercariae from two specimens of *Aequorea eurodina* (Table 2-4). The ITS2 sequences began and ended with the same bases listed for *C. warehou* above. All of the sequences contained complete ITS2 sequences of 293 bases. All sequences were identical except one from a specimen from *S. australasicus*. This sequence differed by two bases. The sequences of digeneans from *S. australasicus* were difficult to obtain, suggesting the DNA may have been slightly degraded.

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Table 2-4 Digenean species, host, locations, replicate information and Genbank accession numbers for trematode sequences

Digenean Species	Host	Locality	No. of hosts from which digenean sequences obtained	No. of digeneans from which sequences obtained	Size (bp)	Accession numbers
<i>Cephalolepidapedon warehou</i>	<i>Cyanea annaskala</i>	Port Phillip Bay	1	3	218, 287, 290	151,142,152,161
	<i>Pseudorhiza haeckeli</i>	Port Phillip Bay	3	4	290	117,118,153,154
	<i>Seriolella brama</i>	Port Phillip Bay	4	6	290	115,116,138,157,158,159
	<i>Seriolella punctata</i>	Off Portland	1	1	290	160
<i>Opechona bacillaris</i>	<i>Aequorea eurodina</i>	Port Phillip Bay	2	2	293	141, 170
	<i>Scomber australasicus</i>	Moreton Bay	2	2	293	130, 150

2.4 Discussion

Digenean metacercariae recovered from three jellyfish species of Port Phillip Bay were identified as two species of lepocreadiid digeneans (*Cephalolepidapedon warehou* and *Opechona bacillaris*) using DNA sequencing. This is the first time DNA sequencing has been used to identify digenean metacercariae in jellyfish. Three new host records for *C. warehou* were recorded, and one new host record for *O. bacillaris*. This discovery also provides potential partial life cycles for two digenean species, including the first intermediate host record for *C. warehou*. The digeneans were identified to species using molecular and morphological methods. Sequences of the digeneans were 99-100% identical to those of adult digeneans recovered from fish known to consume jellyfish. The results from sequencing corresponded with morphological analysis.

2.4.1 *Cephalolepidapedon warehou*

Adult *Cephalolepidapedon warehou* have previously only been reported from the silver warehou, *Seriola punctata* in Tasmania (Bray and Cribb 2003). In the current study, adult *C. warehou* were recorded from *S. punctata* and a new host, the blue warehou, *Seriola brama*. Metacercariae of *C. warehou* parasitised two scyphozoan jellyfish, *Pseudorhiza haeckeli* and *Cyanea annaskala*. This is the first record of *C. warehou* parasitising the fish *S. brama*. This is also the first record of *C. warehou* from jellyfish hosts. It has not been found in any other first (e.g. mollusc) or second intermediate hosts.

The morphology of the metacercariae corresponded to that of the adults, supporting the DNA sequence results. Features that characterise *C. warehou* were found in both adults and metacercariae. These include the funnel-shaped oral sucker, very heavy concentration of pigment between the oral and ventral suckers, the narrow and elongate body and long prepharynx. Forebody, oral sucker width and prepharynx ratios also corresponded between metacercariae and adults.

The equivalence between DNA sequences of *Cephalolepidapedon warehou* adults and metacercariae provided evidence of the species being the same in jellyfish and fish hosts. Aside from two shortened sequences of metacercariae from *Cyanea annaskala*, there was only one base pair in one sequence from an adult digenean that differed to the rest. Interspecific variation in ITS2 sequences is generally substantial (Nolan and Cribb 2005) and while differences of just one base pair between different species have been found (e.g. Agatsuma et al. 2001), this difference was also supported by differing biological characteristics (Greer et al. 1988) and CO1 sequences (Agatsuma et al. 2001). Because

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the sequence difference in the current study only occurred in one of 14 *C. warehou* sequences, and morphological and biological attributes did not differ between the trematodes, the difference is attributed as being due to sequencing error or intraspecific variation rather than a difference in species.

The use of jellyfish as intermediate hosts by *Cephalolepidapedon warehou* is consistent with the close association of the warehouse, *Seriolella brama* and *Seriolella punctata*, with jellyfish. Small juveniles of both species of fish aggregate under jellyfish in Tasmania (Last et al. 1983) and *S. brama* associate with *Catostylus mosaicus*, *Pseudorhiza haeckeli* and *Aequorea eurodina* in Port Phillip (this study and Chapter Five). As adults, *S. brama* and *S. punctata* feed primarily upon pyrosomes, salps and hyperiid amphipods (Bulman et al. 2001; Horn et al. 2011). Unidentified jellyfish were also found in the stomach contents of *S. punctata* (Horn et al. 2011). The high percentage of “unknown” stomach contents recorded in both species of fish (Bulman et al. 2001; Horn et al. 2011) could include jellyfish. Indeed, jellyfish are difficult to identify in gut contents (Arai 1988; Arai et al. 2003), particularly when stomachs are frozen or preserved (Bulman et al. 2001; Horn et al. 2011). An Atlantic Ocean warehouse, *Seriolella porosa*, feeds on ctenophores, making up to 78% of gut contents during summer (Mianzan et al. 1996). Whilst dissecting fish to search for digeneans in this study, hyperiid amphipods (*Hyperia gaudichaudii*) were found in the stomach of an individual *S. punctata*. This amphipod species associates with ctenophores (Zeidler 1992) and *A. eurodina* (this study) and parasitises *P. haeckeli* (this study) and *C. mosaicus* (Chapter Five). As no records of digeneans parasitising salps could be found in the literature, and many other lepopocreadiid species use jellyfish and other gelatinous zooplankton as hosts (e.g. Stunkard 1969, 1980; Køie 1975), it is almost certain that jellyfish act as intermediate hosts for *C. warehou*.

The only other species described for the genus *Cephalolepidapedon* is *Cephalolepidapedon saba* Yamaguti, 1970, which is recorded from chub mackerel, *Scomber japonicus*, blue mackerel, *Scomber australasicus* (Bray and Gibson 1990; Bartoli and Bray 2004), and the jellyfish symbiont butterflyfish, *Psenopsis anomala* (Ohtsuka et al. 2010). These fish feed on gelatinous zooplankton [the mackerel on siphonophores and tunicates (Takano 1954), and the butterflyfish on jellyfish (Suyehiro 1942 cited in Ates 1988)]. *Cephalolepidapedon saba* metacercariae have been found in the scyphozoan jellyfish *Aurelia* sp. (Ohtsuka et al. 2010). The presence of this closely related digenean in both jellyfish and fish that consume gelatinous zooplankton is consistent with the conclusion that *Cephalolepidapedon warehou* use jellyfish as intermediate hosts.

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The presence of *Cephalolepidapedon warehou* metacercariae in jellyfish hosts and mature adults in two fish species which feed on jellyfish or other gelatinous zooplankton (as well as having life-histories closely associated with jellyfish) suggests that *C. warehou* uses the jellyfish *Pseudorhiza haeckeli* and *Cyanea annaskala* as second intermediate hosts. It seems likely *Seriolella brama* and *Seriolella punctata* are infected by eating these scyphozoans or other infected gelatinous zooplankton and act as definitive hosts. This is supported by the similar jellyfish and fish hosts of the closely related *C. saba*. The first intermediate host of *C. warehou* is unknown.

2.4.2 *Opechona bacillaris*

Opechona bacillaris is a widely distributed parasite, reported from warm and cold temperate waters of the Atlantic, Indian, Pacific and Southern oceans (see Bray and Gibson 1990). *Opechona bacillaris* metacercariae have previously been recorded from 11 species of hydrozoan jellyfish, one scyphozoan jellyfish and one ctenophore (Browne unpubl.). This study represents the first record of *O. bacillaris* from the genus *Aequorea*. In addition to gelatinous zooplankton, metacercariae are recorded from planktonic polychaetes (Reimer et al. 1971), chaetognaths (Lebour 1917; Reimer et al. 1971; Køie 1975), free in the plankton (Nicol 1910; Franc 1951), and in fish (families Cyclopteridae, Bothidae, Gadidae and Carangidae, see Bray and Gibson 1990 for references).

The metacercariae of *Opechona bacillaris* from the jellyfish *Aequorea eurodina* had similar features to those of the adults from fish, supporting the DNA sequence results. The characteristic features of the species that occurred in both adults and metacercariae include the elongate oval body, funnel-shaped oral sucker, the oral sucker being larger than the ventral sucker, and the eye-spot pigment in the region of the oral sucker and pharynx. These features were similarly evident in metacercariae from the ctenophore *Pleurobrachia pileus* (Køie 1975). The metacercariae from *A. eurodina* were of a similar size to those recorded by Køie (1975), however, it is difficult to compare measurements as Køie used a different preservative (glutaraldehyde) and did not straighten the specimens (they appear heavily contracted in her photographs and were flattened). Measurements of adult *O. bacillaris* from fish (Bray and Gibson 1990) are also difficult to compare due to the different fixation methods used (Bray and Gibson 1988). However, the forebody of the metacercariae was 44-46% of the body length, much greater than that in the adults (21-36%) (Bray and Gibson 1990). The difference may be a fixing artefact, or as the metacercariae in this study had not developed any sexual organs, the hindbody may expand proportionally in the adult to incorporate these organs.

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The high degree of similarity in the DNA sequences of *Opechona bacillaris* metacercariae and adults provided evidence of the species being hosted by the jellyfish *Aequorea eurodina* and fish *Scomber australasicus*. Indeed, the sequence of *O. bacillaris* from *A. eurodina* was identical to that from one of the *S. australasicus*. The remaining sequence (from a different specimen of *S. australasicus*) differed from these sequences by only two base pairs (of 293). The two base differences were due to adenine-guanine transitions. At one of the differing base pair positions, the peak of the adenine was almost as high as the guanine peak characterised by the sequence. The inconsistent sequence had a low concentration and the reverse sequence was of poor quality. It seems likely, therefore, that these base differences may be due to sequencing error, probably from degraded tissue caused by freezing of the samples prior to extracting the digeneans, rather than due to intraspecific variation. Degradation and enzymatic activity may occur at -20°C (Nagy 2010) and frozen thawed material is not recommended to be used (Justine et al. 2012). Differences of 2 base pairs in the ITS2 region have been interpreted as two different species (Miller et al. 2009), however, these were supported by relatively large morphological disparity, and consistent genetic differences in ITS2 (2 base pairs) and ITS1 (3 bp). To confidently ascribe the differences in the sequences from the current study as due to sequence error, intra- or interspecific variation, further replication of samples from fresh digeneans and sequencing of different regions would be necessary. However, for the purpose of this study, the 100% match of sequences from digeneans hosted by an *A. eurodina* and a *S. australasicus* combined with the morphological similarities and biological evidence were deemed sufficient to identify the species from both these hosts as *O. bacillaris*.

The definitive hosts of *Opechona bacillaris* are predominately fish of the family Scombridae: *Rastrelliger brachysoma*, *Rastrelliger kanagurta*, *Scomber australasicus*, *Scomber japonicus* and *Scomber scombrus* (see Bray and Gibson 1990 for references); but also include fish from 13 other families (see Bray and Gibson 1990). This study is the second report of *O. bacillaris* from *S. australasicus*, the previous being from the Great Australian Bight (Korotaeva 1974). High prevalences (45-100%) of *O. bacillaris* have been found in *S. australasicus* (45.2% of 42 fish) (Korotaeva 1974), *S. scombrus* (Nicoll 1910) and *S. japonicus* (Hadzhiiski 1980 cited in Bray and Gibson 1990), suggesting these are important hosts of the trematode. *Scomber scombrus* is restricted to the North Atlantic Ocean (Collette 2003), while the range of *S. japonicus* overlaps with that of *S. australasicus* in the Indo-Pacific Ocean (Collette 2001). *Scomber australasicus* is the only *Scomber* species found in Australian waters (Gomon et al. 2008), and one of only two

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known fish hosts of *O. bacillaris* in Australian waters. The other host is *R. kanagurta*, which is distributed in the tropical Indo-west Pacific including Western Australia, the Northern Territory and north Queensland (ABRS, 2014). Therefore, in the regions from which *O. bacillaris* were obtained for this study, *S. australasicus* is the most likely definitive host.

Scomber australasicus are omnivores that feed primarily upon pelagic ascidians, pyrosomes and salps, and also in lesser amounts of fish and crustacea (Bulman et al. 2001). *Scomber japonicus* and *S. australasicus* also feed upon the siphonophore *Chelophyes appendiculata* (Takano 1954). *Scomber scombrus* feeds upon the hydromedusae *Aglantha digitale* (Runge et al. 1987). Given the broad diet range (including gelatinous zooplankton) of *S. australasicus* and its congeners, it seems likely that *S. australasicus* feeds upon *Aequorea eurodina*. This information combined with the finding of *Opechona bacillaris* in *A. eurodina* in this study, suggest that *O. bacillaris* uses *A. eurodina* as an intermediate host, and *S. australasicus* as a definitive host. The range of animals in which *O. bacillaris* metacercariae and sexual adults have been found, suggests there may be other second intermediate (and definitive) hosts in the Pacific. The prosobranch *Nassarius pygmaeus* is the first intermediate host in Hornbaek Bay in the Øresund, Denmark (Køie 1975). Species of *Nassarius* are found along most of the Australian coastline (ABRS 2014), so it would be an appropriate genus for further investigation into the life cycle of *O. bacillaris*.

2.4.3 Implications of findings

Seriolella punctata, *S. brama* and *Scomber australasicus* are all commercially harvested. The warehouse, *S. brama* and *S. punctata*, had annual catch values in Australia (2009-2010) of at least \$AUD 0.222 million, and \$AUD 3.37 million respectively (ABARES, 2011). *Seriolella punctata* is a particularly important trawl species in the Southern and Eastern Scalefish and Shark Fishery trawl, making up 8.9% of the catch weight (ABARES, 2011). In addition, *S. brama* and *S. australasicus* are caught by recreational fishers (Kailola et al. 1993; Ward et al. 2009). *Scomber australasicus* forms important fisheries in China, Korea, Japan (Yukami et al. 2009), Taiwan (Tzeng 2004) Australia and New Zealand (Collette 2001). In Japan for example, the annual catch for 2006 was approximately 59 000 tons (Yukami et al. 2009). The discovery of *Cephalolepidapedon warehou* in *S. brama* and the confirmation of *C. warehou* in *S. punctata* and *O. bacillaris* in *S. australasicus* provide valuable information for these fisheries. Accurate identification of fish parasites is fundamental for fisheries and aquacultural development, management and sustainability (Catalano et al. 2011). In addition, knowledge of the life cycles of fish parasites provides

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important information about linkages within the marine environment (e.g. Thompson et al. 2005). Fish parasites may be used to provide information about diet (Williams et al. 1992), populations (MacKenzie 2002), migrations (e.g. Carballo et al. 2012), phylogenies (Xiao et al. 2001) and environmental changes (Palm 2011).

There have been no studies of the effects of *Opechona bacillaris* or *Cephalolepidapedon warehou* on their fish hosts. However, trematodes such as these, found in the intestines of their definitive hosts, are generally not considered significant pathogens (Cribb 2005). These digeneans were small in comparison to the fish studied and did not occur in massive intensities. Although intestinal digeneans may feed on food within the host's intestines, and not directly damage the host, they may still reduce fitness of the host fish through energy loss and increased feeding effort (Bartoli and Boudouresque 2007). The effects of the digenean parasites may be greater upon hosts other than fish within their life cycle.

No obvious effects of the digeneans were observed on the jellyfish. This agrees with observations by Køie (1975) on most ctenophores and hydromedusae infected by *Opechona bacillaris*. However, she found that very small hydromedusae *Hydractinia carnea* were seriously affected and unable to swim when penetrated by four cercariae. She did not give sizes of the hydromedusae, however, large *H. carnea* medusae have a bell diameter of only 2.4 mm (Schuchert 2008), much smaller than the jellyfish in this study. Populations of the ctenophore *Pleurobrachia pileus* declined after heavy infection by *O. bacillaris* and didymozoid trematodes (Yip 1981; Yip 1984), suggesting that heavy infections can affect secondary intermediate jellyfish hosts. The hosts most affected by *O. bacillaris* and *C. warehou* are likely to be the first intermediate hosts, which typically have their health harmed by digeneans (Cribb 2005). While there are no studies on first intermediate hosts of *C. warehou*, male gastropods (*Nassarius pygmaeus*) infected with *O. bacillaris* cercariae had a highly reduced mating organ, and infected males and females had a reduced and non-functional gonad (Køie 1975). Similarly, gastropods parasitised by lepopocreadiid cercariae believed to be *Opechona* sp. were completely castrated (Averbuj and Cremonte 2010). As prevalences of infected snails may be high (e.g. 7.4% of *Nassarius pygmaeus* in Køie's study, up to 54.2% of *Buccinanops cochlidium* in Averbuj and Cremonte's), infections could have effects on a population level.

This study determined new jellyfish hosts for the lepopocreadiid trematodes *Cephalolepidapedon warehou* and *Opechona bacillaris*. Sequences of ITS2 proved to be an effective tool in identifying digenean metacercariae from jellyfish. Results from

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morphology, ecological data and previous studies suggest that these jellyfish act as second intermediate hosts to these digenean species. A new definitive host for *C. warehou* has been determined (*Seriolella brama*) and definitive hosts of both trematode species are further supported (*C. warehou* in *Seriolella punctata* and *O. bacillaris* in *Scomber australasicus*).

3 Spatial variation of endoparasites of *Cassiopea* sp.

3.1 Introduction

Spatial variation in marine endoparasites is linked to many factors operating at different scales. Differences occur across latitudes (Rohde and Heap 1998), and may relate to differences in the availability (Detwiler and Minchella 2009) or diversity (Hechinger and Lafferty 2005) of hosts. Indeed, for parasites to complete their life cycle, the first intermediate and definitive hosts must occur within the range of the second intermediate host (or infective parasite stages) for hosts to be infected. Therefore, variability in the availability of hosts is probably important in determining the spatial distribution of jellyfish endoparasites. The diversity of free-living animals is related to latitude, with the increase in richness of free-living species from the poles to the equator recognised as a fundamental distribution pattern (Rohde 1992; Hilebrand 2004; Thieltges et al. 2009b). This gradient has similarly been identified in some marine parasites (e.g. fish ectoparasites, Poulin and Rohde 1997; Rohde and Heap 1998) but not others (e.g. fish endoparasites, Rohde 2002).

The parasite communities of many aquatic hosts are structured so that nearby communities are more similar than those further apart (Poulin et al. 2011; Santana-Piñeros et al. 2012). This spatial structure is a reflection of Tobler's first law of geography "everything is related to everything else, but near things are more related than distant things" (Tobler 1970). Studies of the spatial structure of the endoparasites of marine intermediate hosts are rare and have not been carried out for jellyfish acting as intermediate hosts.

Digeneans and cestodes parasitise jellyfish (e.g. Martorelli 2001; Diaz Briz et al. 2012) and jellyfish are probably important intermediate hosts for these taxa. Most studies of endoparasites in jellyfish have focused on taxonomy (e.g. Reimer 1976; Stunkard 1980b; Martorelli 2001) and quantifying the prevalence and intensity of infections (e.g. Morandini et al. 2005) and studies of the ecology of the parasitic association between jellyfish and their endoparasites are rare. Indeed only Diaz Briz et al. (2012) have examined spatial variability in the prevalence of digenean parasites in medusae in the coastal waters of the southwest Atlantic Ocean. They observed greater prevalence in estuaries and in the North Patagonian tidal front, which they suggested was due to the oceanographic front concentrating medusae and fish, thereby facilitating the transmission of parasites between hosts. In addition to spatial variability, parasite communities may also vary through time.

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For example, seasonal variability in prevalence but not intensity of infection by the digenean *Opechona pyriforme* in the hydromedusa *Eirene tenuis* was observed and was postulated to relate to changes in salinity or temperature (Martell-Hernández et al. 2011). The congener *Opechona bacillaris* also showed strong seasonal variation in rates of infection in the ctenophore *Pleurobrachia pileus* in Galway Bay, Ireland (Yip 1984). Understanding the spatial and temporal variation in infection parameters of endoparasites will help elucidate the population dynamics of the parasites and may improve understanding of how the parasite may affect host populations.

Cassiopea is an unusual genus of scyphozoan because they live a semi-sessile existence, positioned on the benthos with their zooxanthellate oral arms facing upwards (Figure 3-1). *Cassiopea* occur in shallow tropical, and sub-tropical marine waters globally, on mud flats, coral reefs, seagrass meadows, reef-sand transition habitats and sand flats (Holland et al. 2004; Niggli and Wild 2010). Symbionts of *Cassiopea* include the dinoflagellate *Symbiodinium* (Sachs and Wilcox 2006; Lampert et al. 2012), the pontoninid shrimp *Periclimenes holthuisi*, the mysid shrimp *Idiomysis tsumamali* (Băcescu 1973; Niggli and Wild 2010), and the copepod *Sewellocchiron fidens* (Humes 1969 cited in Humes 1985) but no parasites have been reported.



Figure 3-1 *Cassiopea* sp. in its customary position on the seafloor. Photograph taken at Lizard Island

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The aims of this study were to:

- Compare the diversity, intensity and prevalence of endoparasites infecting *Cassiopea* sp. at four locations across a large spatial scale (approximately 13° latitude);
- To examine variation in the location of endoparasites within individual animals;
- To determine whether significant variation occurred in the endoparasite communities of *Cassiopea* sp. at a single location over two time periods; and
- To use molecular techniques to identify endoparasite species and compare the DNA sequences of morphotypes.

3.2 Methods

3.2.1 Study animal

A molecular global phylogeography of *Cassiopea* (Holland et al. 2004) indicates the species for the present study is likely to be *Cassiopea* sp. 1. As no molecular analysis of the jellyfish was undertaken, and identification to species level within *Cassiopea* using morphology is difficult, and has a history of confusion and disagreement (Holland et al. 2004), in this study the name *Cassiopea* sp. is used.

3.2.2 Study Locations

Cassiopea sp. were sampled at four locations over an approximately 1600 km range, within Queensland, Australia (Figure 3-2, Table 3-1). Jellyfish were collected from shallow sand or silt flats at all sites. They were gently scooped into a zip-lock bag by hand (Figure 3-3), either on SCUBA or by wading from the shore. The two northernmost sites, Lizard Island and Vlasoff Reef (Figure 3-4), are part of the Great Barrier Reef (GBR). Lizard Island (14°41'18.02"S, 145°27'32.22"E) is a continental island located approximately 30 km offshore with fringing reefs and a large lagoon. Vlasoff Reef (16°39'0.83"S, 145°59'21.30"E) is approximately 24 km from mainland Australia and is part of the Arlington Reef complex which consists of patch reefs and small reef-cay complexes (Alongi et al. 2006). Lake Magellan (26°49'33.35"S, 153°6'53.89"E, Figure 3-4) is a shallow (<3 m) artificial lake within a residential development separated by a piped outlet to Lamerough Canal which flows into Pumicestone Passage. Crab Island (Figure 3-4) is a low-lying, mangrove-covered island (27°20'39.26"S, 153°24'17.10"E), just 65 km SSW of Lake Magellan) located on the eastern edge of Moreton Bay, a sub-tropical, shallow coastal embayment. *Cassiopea* sp. were collected from a shallow, hypersaline (40 ppt)

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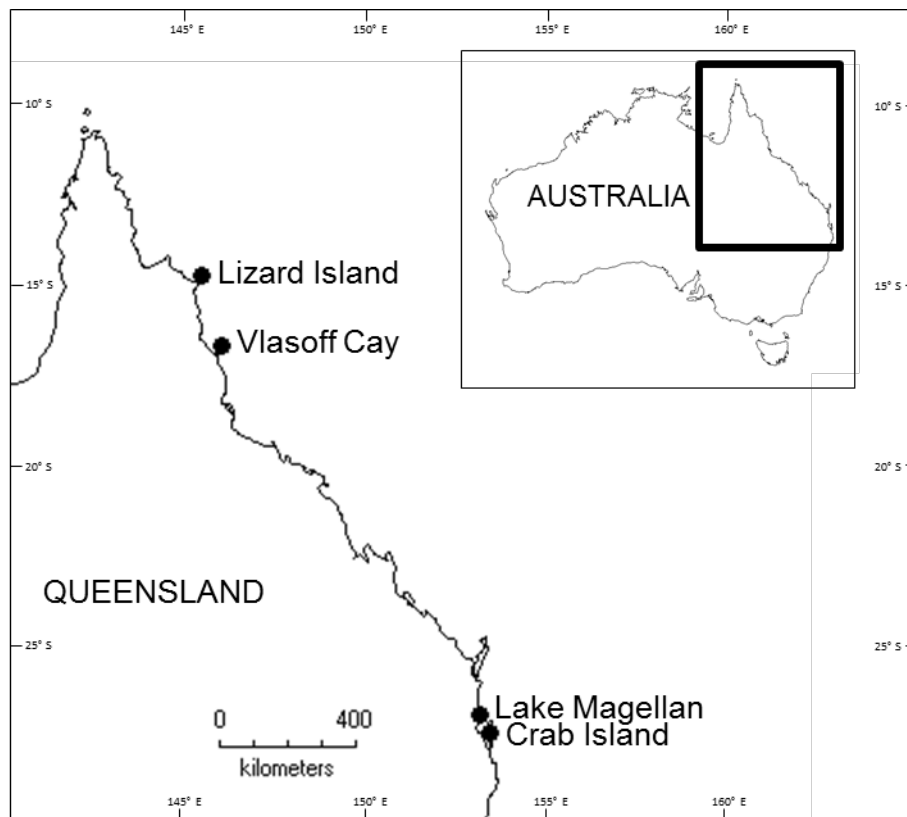


Figure 3-2 Locations at which *Cassiopea* sp. were collected



Figure 3-3 *Cassiopea* sp. being scooped into a zip lock plastic bag. Photo: Kade Mills

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Figure 3-4 Images of (a) Lizard Island, (b) Vlasoff Reef, (c) pool at Crab Island and (d) Lake Magellan

Table 3-1 Collection dates, initial number and bell diameters of *Cassiopea* sp. collected, depth from which they were collected, and collection method for each location

Location	Collection dates	Number of <i>Cassiopea</i> sp. initially collected	Bell diameter of dissected specimens (cm)	Depth of collection (m)	Collection method
Lizard Island	15-20 Apr 2008	12	1.5-6	9	SCUBA
	23-25 Feb 2009	35	3.1-6	9	SCUBA
Vlasoff Cay	21-22 Jan 2009	30	2.5-5	7	SCUBA
Lake Magellan	18 Mar 2009	70	3-8	0.5-0.7	hand
Crab Island	17 Mar 2009	73	3-7	0.5	hand

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lagoon that formed in the centre of the island due to the dieback of mangroves (Duke et al. 2010). The lagoon had an anoxic odour and a layer of microalgae covered the silty bottom. The lagoon has no direct connection to the sea.

3.2.3 *Treatment of specimens*

Cassiopea sp. were examined briefly for commensals under a dissecting microscope in the laboratory whilst still alive and wet weight and bell diameter were measured. All jellyfish collected from Lizard Island in 2008 were preserved in formalin. In 2009, half the jellyfish from each location were preserved in 5% formalin buffered with borax and the remaining half were preserved in 96% ethanol. From these preserved specimens, fifteen from each location within the size range 2.5-8 cm were dissected. The bell of each jellyfish was separated from the oral arms and disc and the different tissues were examined for the presence of parasites using a Leica Wild M8 stereomicroscope. The position of each parasite within the jellyfish was recorded. Position was categorised as being in the centre, middle or outer edge of the bell and oral disc; or the proximal, middle or distal ends of the oral arms. The parasites were removed and assigned to a morphotype. They were then preserved in 10% formalin or 96% ethanol, consistent with the preservative used for their host jellyfish.

For all parasites collected in 2009 each individual was placed on a concave slide and viewed using an Olympus BX50 compound microscope (except for Morphotype A for which 22 individuals were examined). Encysted digeneans were manually excysted using hypodermic needles but unfortunately most of the specimens were damaged in this process due to the minute size of many of the digeneans (<150 µm). Excysting was presumed to allow maximum digestion of samples for DNA analysis and to aid identification of morphological specimens. Photographs were taken with a QImaging Go-21 CMOS camera mounted on the microscope and measurements were made using an ocular micrometer. The length and width of each parasite was measured. In addition, the following morphological characters were measured for digeneans: length and width of cyst, length and width of digenean in cyst, length of forebody, oral sucker width, oral sucker length, ventral sucker width and ventral sucker length. Photographs taken at varying focal lengths were stacked using ImageJ freeware (Schneider et al. 2012) and were all entered and categorised using IMatch, a digital asset management system. Images (814 in total), measurements and specimens were used to determine morphotypes.

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Digeneans preserved in ethanol were sequenced for DNA (see Chapter Two). An adult digenean specimen from the collection of Dr Thomas H. Cribb (University of Queensland, Australia) was also sequenced. These specimens were selected from a fish host suspected to be a jellyfish predator (*Naso vlaminghii*). Some cestode specimens were sent to a cestode taxonomist (Professor Ian Beveridge, University of Melbourne, Australia) for identification.

Digeneans were categorised into morphotypes, rather than species due to the difficulty of identifying the metacercaria stage which occurs in jellyfish. Encysted metacercariae are particularly difficult to identify as they must first be excysted. This is most reliably done through chemical excystment which requires the digenean to be alive (e.g. Fried 1994). Endoparasites were unable to be removed from jellyfish while alive due to the opacity of the *Cassiopea* sp. caused by the presence of zooxanthellae throughout the mesoglea of the jellyfish. After preservation in ethanol or formalin, endoparasites were able to be seen. Cestodes are also difficult to identify in their larval form. Identification of cestodes through DNA sequencing was not attempted due to a low probability of success as there are few sequenced adult cestodes from likely definitive hosts from the regions sampled.

3.2.4 Statistical analysis

Infection parameters (prevalence, mean intensity, range and abundance) were calculated according to Bush et al. (1997) (see Glossary). Differences in parasite community composition between the different locations were graphically displayed using non-metric multi-dimensional scaling (MDS) based on a zero-adjusted Bray-Curtis similarity matrix (Bray and Curtis 1957). Prior to this, the data were square-root transformed to downweight the importance of highly abundant species. The Bray-Curtis matrix was zero-adjusted (by the addition of the constant 1), due to the large numbers of hosts which had zero or few parasites (Clarke et al. 2006). The number of restarts for the MDS was 50, otherwise defaults were used. To test for differences between Lizard Island and Vlasoff Reef, analysis of similarity (ANOSIM) procedures were used (Clarke 1993) on the zero-adjusted Bray-Curtis matrix. Only the two locations were selected due to the low abundance of parasites of *Cassiopea* sp. at Lake Magellan and Crab Island. ANOSIM provides a global *R* value based on average similarities within replicate samples, and average similarities between different samples. The global *R* value is tested by evaluating random permutations of the data; this was repeated 999 times in this instance. All multivariate analyses were undertaken using PRIMER 6, version 6.1.13 (Clarke and Gorley 2006).

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Pearson's correlation was used to determine whether a correlation existed between *Cassiopea* sp. bell diameter and weight.

Two, one-way analyses of variance (ANOVAs) were used to test for differences in intensity of infection of digeneans between a) Lizard Island and Crab Island and b) the intensity of encysted digeneans at two times sampled at Lizard Island. Prior to analyses, a Cochran's test was used to test data for homogeneity of variances. If heterogeneous, the data were transformed using a $\log_e(x + 1)$ transformation (the location comparison). If the test was still significant after all appropriate transformations were attempted (the location comparison), analyses were still undertaken on transformed data as ANOVA is fairly robust to non-normality, particularly where replication is large and samples are balanced (Underwood 1997). In this case, if data was highly skewed $\log_e(x + 1)$ was used as it removes the skewness and increases normality of the data (Underwood 1997).

3.2.5 *Phylogenetic analysis*

A single sequence for each specimen was produced using methods from Chapter Two. Sequence data were subjected to BLAST searches and also compared with sequence data from the authors' unpublished databases. Sequences were selected from BLAST only if the Expectation (E) value was $<10^{-3}$ and query coverage was $\geq 60\%$. Sequences were aligned, checked and trimmed and distance matrices constructed as per methods in Chapter Two.

3.3 Results

3.3.1 *Morphotype Diversity*

A total of 72 *Cassiopea* sp. were examined, 12 from the 2008 collection, and 60 from 2009. From jellyfish collected in 2009, 23 metazoan endoparasite morphotypes were found, represented by one larval cestode (tentatively identified as belonging to the order Trypanorhyncha), 15 encysted digenean metacercariae morphotypes, 6 unencysted digenean metacercariae morphotypes and 2 unidentified endoparasites (Figure 3-5, Table 3-2). There was little overlap of morphotypes between locations (Table 3-2, Figure 3-6), with only one cestode morphotype and two digenean metacercariae morphotypes found at both Lizard Island and Vlasoff Reef. Although Lizard Island and Vlasoff Reef shared three morphotypes, endoparasite communities differed significantly between Lizard Island and Vlasoff Reef (ANOISIM: $R = 0.778$, $p < 0.001$). Lizard Island showed the greatest diversity

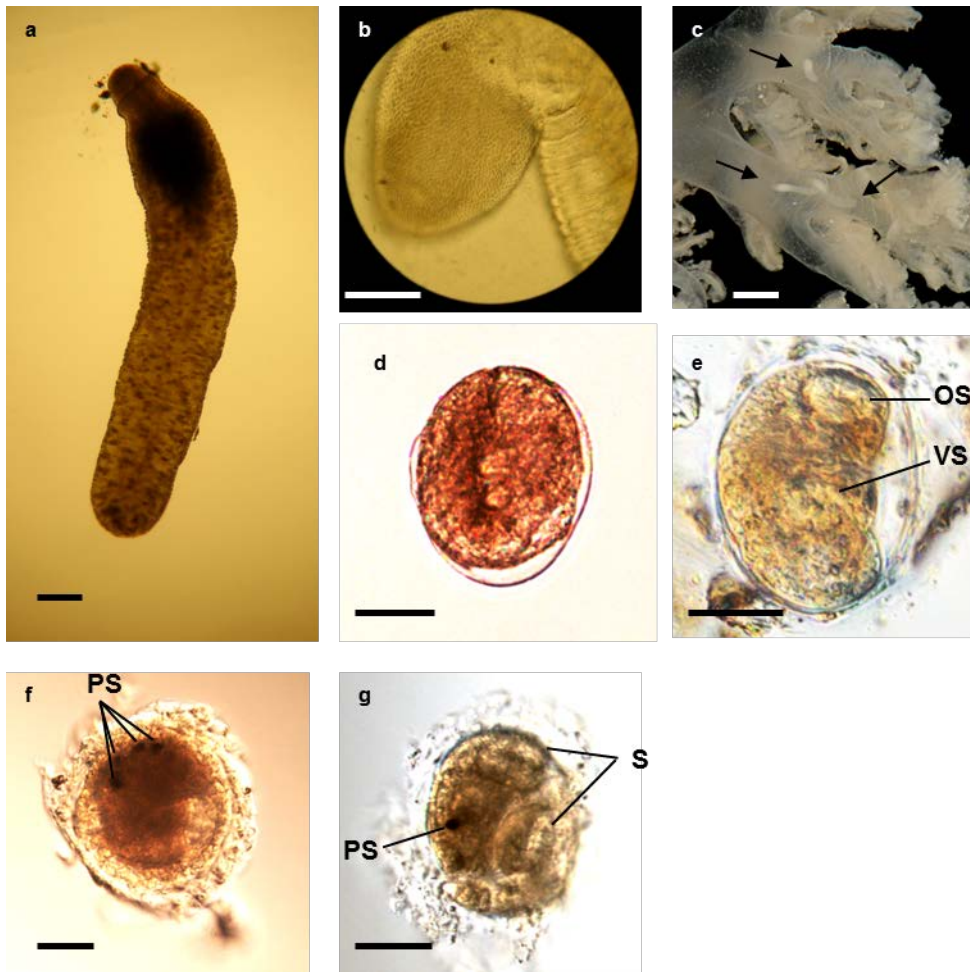


Figure 3-5 Selected parasites from *Cassiopea* sp. a-c: trypanorhynch metacestodes (b) detailed view of scolex (c) *in situ*, (d) morphotype A, (e) morphotype B, (f) morphotype F, (g) morphotype I. Scale bar = 50 μ m except (a) 200 μ m, (c) 2 cm. OS = oral sucker, PS = pigment spots, S = sucker, VS = ventral sucker. Morphotype B selected for inclusion in figure as it clearly shows OS and VS. Other morphotypes selected as their DNA was analysed.

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Table 3-2 Prevalence (P), mean intensity and range for each parasite/commensal morphotype at each location. (n = 15 for each location)

	Lizard Island		Vlasoff Reef		Lake Magellan		Crab Island		DNA sequence obtained (no of specimens)
	P (%)	Mean Intensity (range)	P (%)	Mean Intensity (range)	P (%)	Mean Intensity (range)	P (%)	Mean Intensity (range)	
Cestoda									
Trypanorhynch metacestode	20	1 (1-2)	40	1 (1-3)					
Digenea									
Encysted metacercariae									
Type A	93	9 (2-22)	13	1 (1)					3
Type B			33	1 (1)					
Type C	7	1 (1)							
Type D	7	1 (1)							
Type E	7	1 (1)							
Type F							13	3 (2-4)	1
Type G					7	1 (1)			
Type H	27	1 (1-2)	20	1 (1)					
Type I	7	1 (1)							1
Type J	7	1 (1)							
Type K			40	1 (1-2)					
Type L	20	1 (1-2)							
Type M	27	1 (1-2)							
Type N	7	1 (1)							
Unencysted metacercariae									
Pseudopisthgonoporus vitellus					7	2 (2)			1
Type O	7	1 (1)							
Type P	13	1 (1)							
Type Q	7	1 (1)							
Type R	7	1 (1)							
Type S	27	2 (1-3)							
Copepoda									
Cyclopoid copepod	27	3 (1-4)	20	1 (1)					
Unidentified endoparasites									
unidentified 1							13	2 (1-2)	
unidentified 2	7	1 (1)							

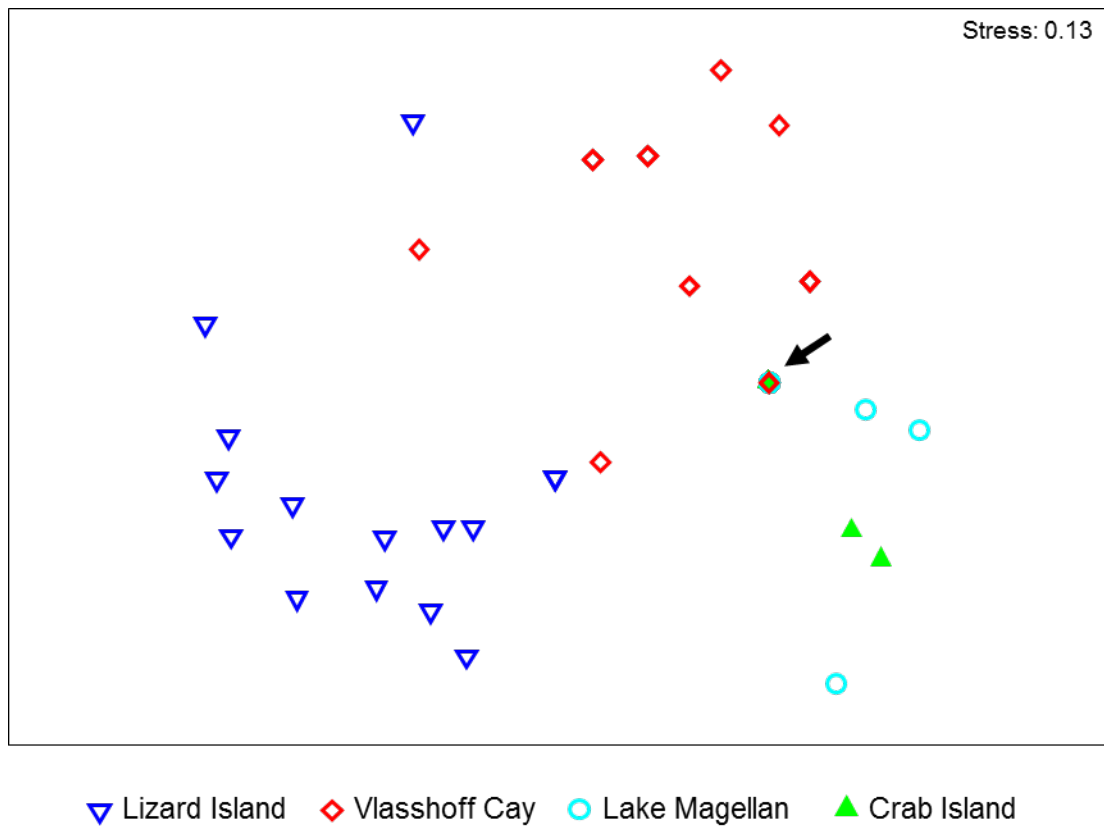


Figure 3-6 MDS plot showing differences in assemblages of endoparasites in *Cassiopea* sp. at four locations. Each symbol represents the endoparasite community in individual jellyfish. The degree of similarity between assemblages is indicated by the proximity. The arrow points to the symbols representing the jellyfish with no endoparasites

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of endoparasites with 17 morphotypes, Vlasoff Reef had six, and Lake Magellan and Crab Island each had only two. One species of commensal copepod was also found attached to or swimming around the jellyfish (Table 3-2) at Lizard Island and Vlasoff Reef.

3.3.2 *Infection parameters*

The bell diameter of *Cassiopea* sp. was strongly correlated with *Cassiopea* sp. wet weight ($n = 45$, $r = 0.96$, $p < 0.01$, Figure 3-8). Therefore bell diameter was used as an indicator of host size. There was some variation in the size distribution of jellyfish sampled at each location (Figure 3-7). However, as there was no clear relationship between bell diameter of *Cassiopea* sp. and total digenean intensity at Lizard Island and Crab Island (Figure 3-9), statistical comparison of mean intensities of digeneans at these two locations was considered reasonable. The abundances of parasites at Crab Island and Lake Magellan were too low to determine whether there was a relationship between bell diameter and intensity.

Cestodes were only found at Lizard Island and Vlasoff Reef. The mean intensity and range were low at each location (Table 3-2). Prevalence was twice as high (40%), at Vlasoff Reef as at Lizard Island (20%).

Mean intensity of total digeneans in *Cassiopea* sp. differed between Lizard Island and Vlasoff Reef (ANOVA: $F_{(1,28)} = 53.2$, $P < 0.001$). Intensity was highest at Lizard Island, lowest at Vlasoff Reef and intermediate at both Lake Magellan and Crab Island (Figure 3-10a). The high mean intensity of digeneans at Lizard Island was primarily due to the digenean Morphotype A which had a range in intensity from 2-22 individuals per jellyfish (Table 3-2). This digenean was also present at Vlasoff Reef, although it attained a maximum intensity of only 1 individual per jellyfish there (Table 3-2). Apart from Morphotype A, the mean intensity of endoparasites was low for each morphotype (between 1 and 3, Table 3-2). Prevalences of total digeneans were much higher at the GBR sites Lizard Island and Vlasoff Reef, than at Lake Magellan and Crab Island (Figure 3-10b). Morphotype A was the most prevalent morphotype, found in 93% of jellyfish. Prevalences of many of the digenean morphotypes were low, with eleven of the morphotypes each only found in one jellyfish (7% prevalence; Table 3-2). Of these eleven morphotypes, ten were represented by only one specimen. The digeneans were highly aggregated among the sampled jellyfish with most jellyfish having no digeneans, and some hosting many (Figure 3-11). The distribution followed a negative binomial distribution.

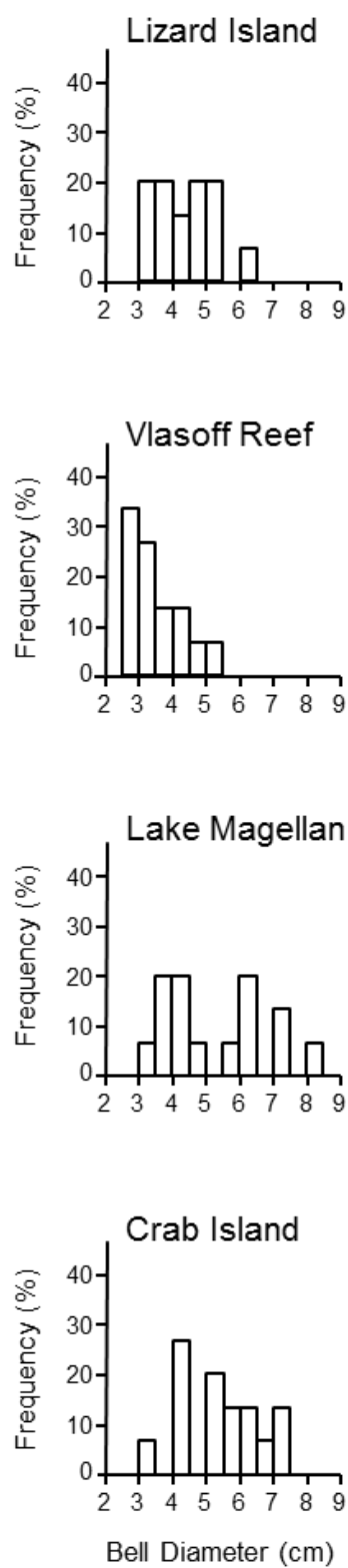


Figure 3-7 Size-frequency distribution of sampled *Cassiopea* sp. using bell diameter (n=15)

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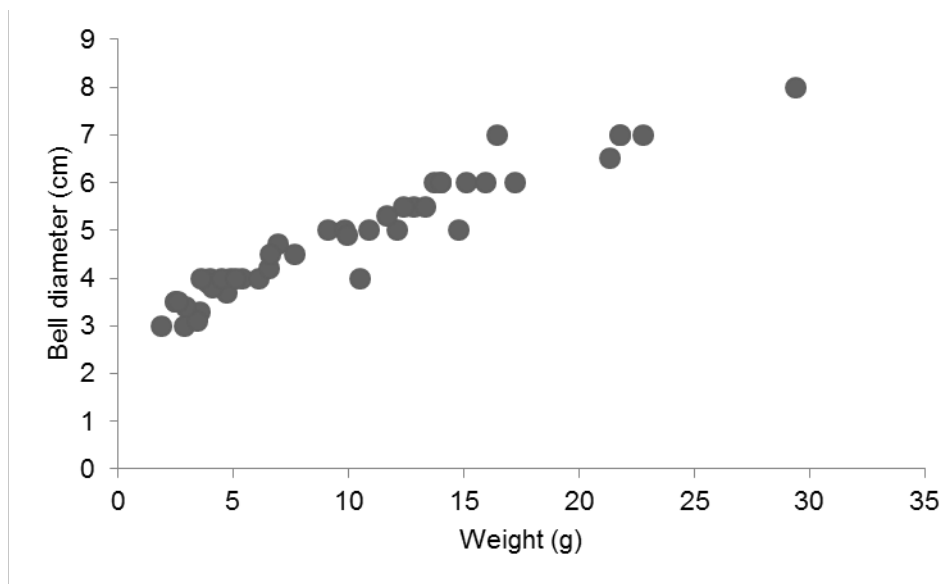


Figure 3-8 Bell diameter versus weight for all sampled *Cassiopea* sp. (n=45)

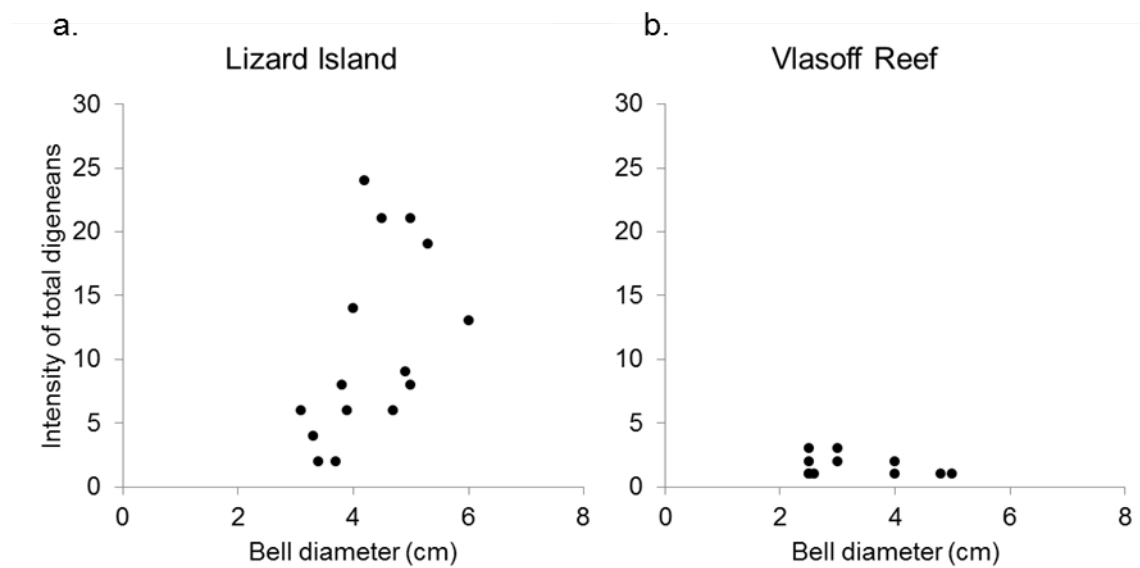


Figure 3-9 Intensity of total digeneans versus bell diameter of *Cassiopea* sp. at (a) Lizard Island and (b) Vlasoff Reef

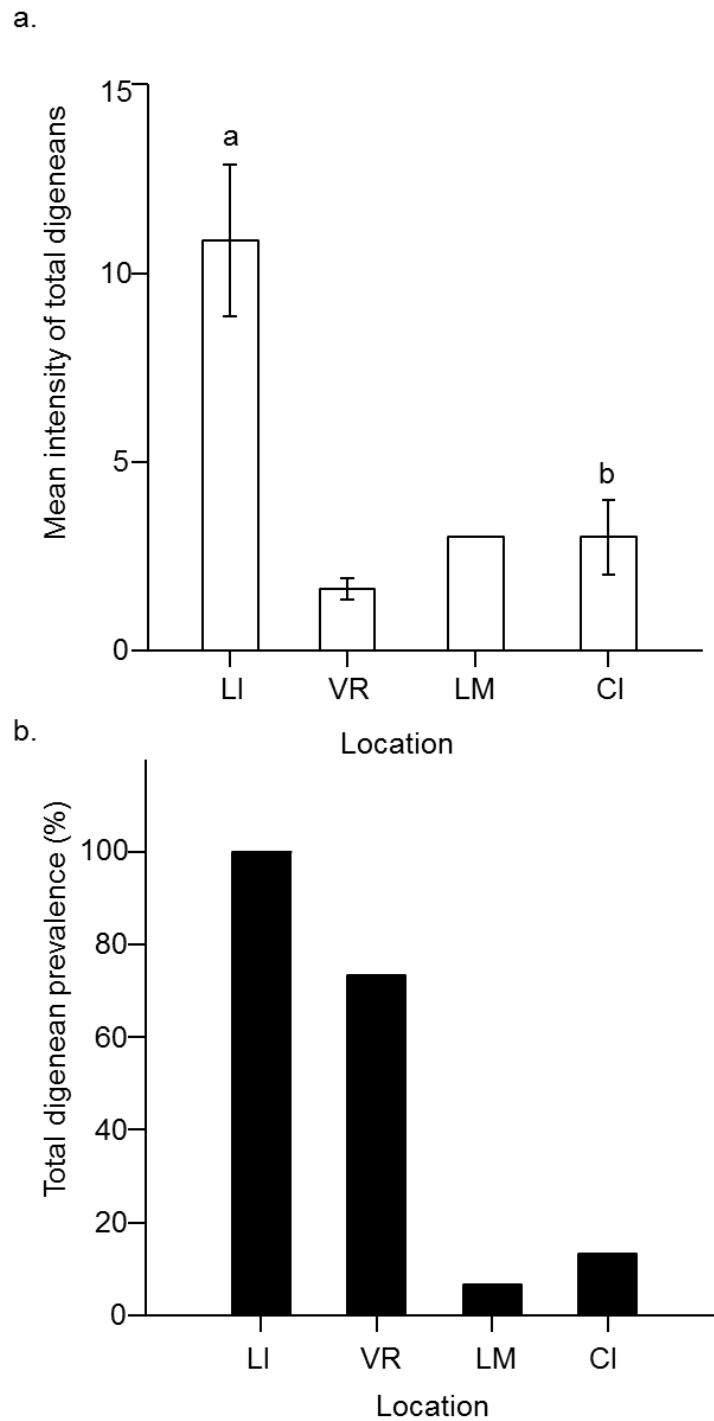


Figure 3-10 (a) Mean intensity of total digeneans at each location (error bars indicate standard error; different letters denote significant differences as tested by ANOVA, note ANOVA was only used for Lizard Island and Vlasoff Reef) (b) Prevalence of total digeneans at each location. Locations in (a) and (b) are Lizard Island (LI), Vlasoff Reef (VR), Lake Magellan (LM) and Crab Island (CI)

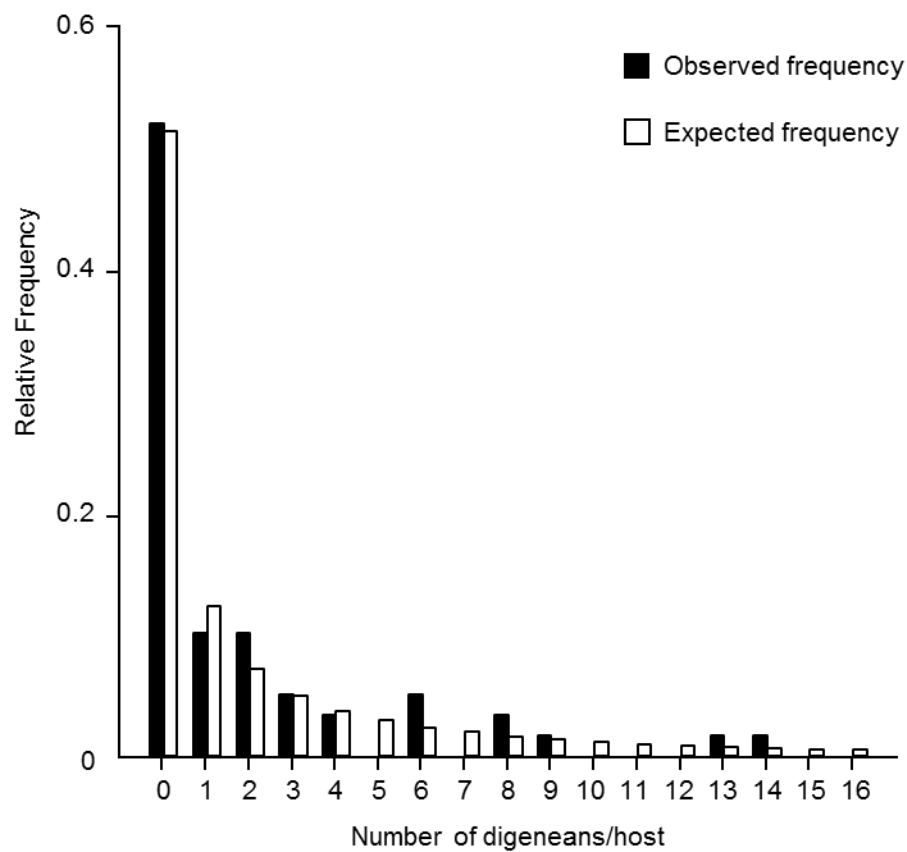


Figure 3-11 Frequency distribution of the number of digeneans per *Cassiopea* sp. (n=60). Expected frequencies according to the negative binomial model

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3.3.3 *Position of Endoparasites within Cassiopea sp.*

The majority of cestodes occurred in the oral arms of *Cassiopea* sp., and all of these occurred at the distal ends of the arms (Figure 3-12a). The remainder were found in the middle or outer edges of the bell and oral disc (Figure 3-12a).

Digeneans were also mostly found within the oral arms of *Cassiopea* sp., with most of these at the distal ends, followed by the proximal ends (Figure 3-12b). Digeneans also occurred in the bell and oral disc of the jellyfish, in higher numbers at the outer edge of both of these, than the centre or middle (Figure 3-12b). No parasites were observed in gonadal tissue.

3.3.4 *Temporal variation at Lizard Island*

Because the 2008 samples were not identified to morphotypes, variation between endoparasites sampled during 2008 and 2009 at Lizard Island was assessed based on the following categories: cestodes, unencysted digeneans, and encysted digeneans. The prevalence and mean intensity of endoparasite groups of *Cassiopea* sp. at Lizard Island between April 2008 and February 2009 was similar for each group (Figure 3-13a). There was some variation in size in the size range of range of *Cassiopea* sp. between years (Table 3-1). As there was no clear relationship between the bell diameter of *Cassiopea* sp. and encysted digenean intensity in 2008 or 2009, the mean intensities of encysted digeneans were compared statistically. The abundances of cestodes and unencysted digeneans were too low to determine whether there was a relationship between bell diameter and intensity. The mean intensities of encysted digeneans were not significantly different between years (Figure 3-13a, ANOVA: $F_{(1,22)} = 0.40$). Prevalence was higher for both categories of digeneans in 2009 (Figure 3-13b). The mean intensity and prevalence of cestodes was slightly higher in 2008 than 2009 (Figure 3-13).

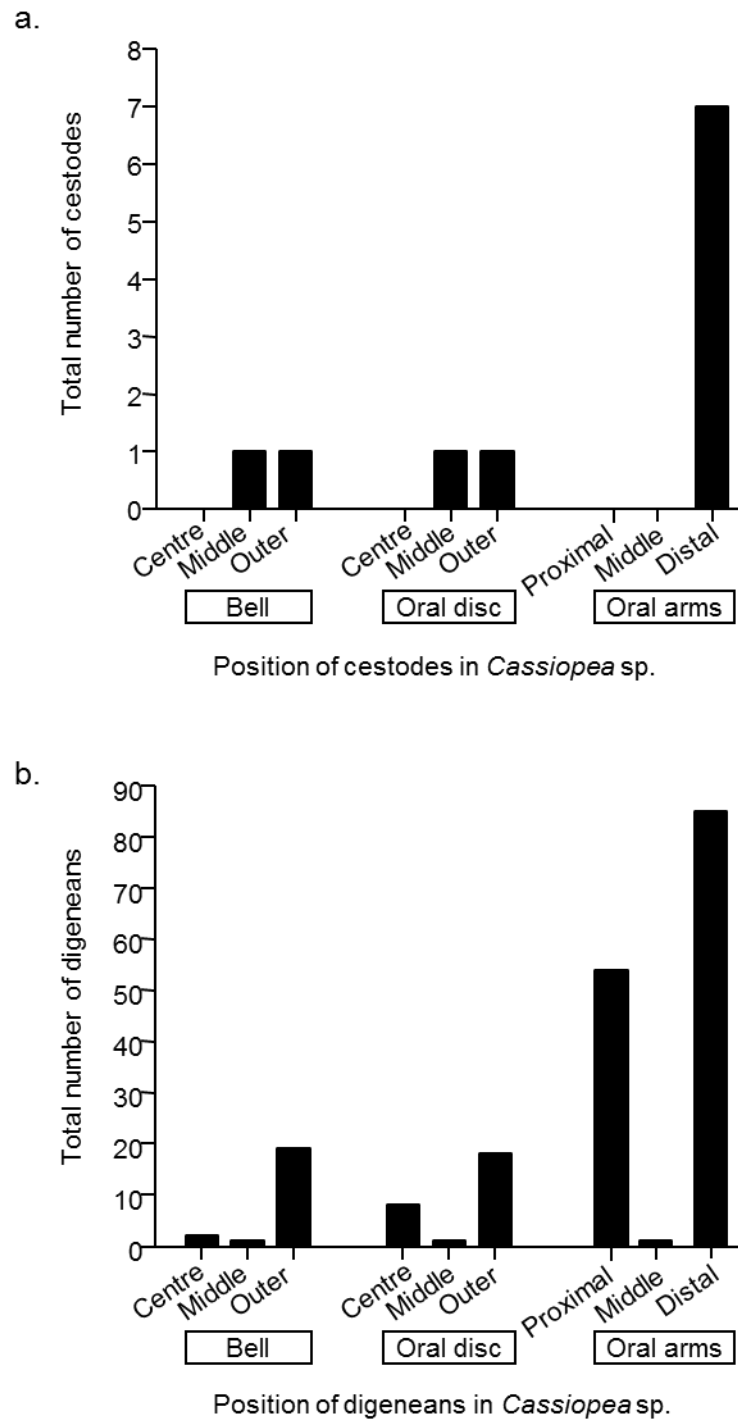


Figure 3-12 Position of (a) cestodes and (b) digeneans within body of *Cassiopea* sp.

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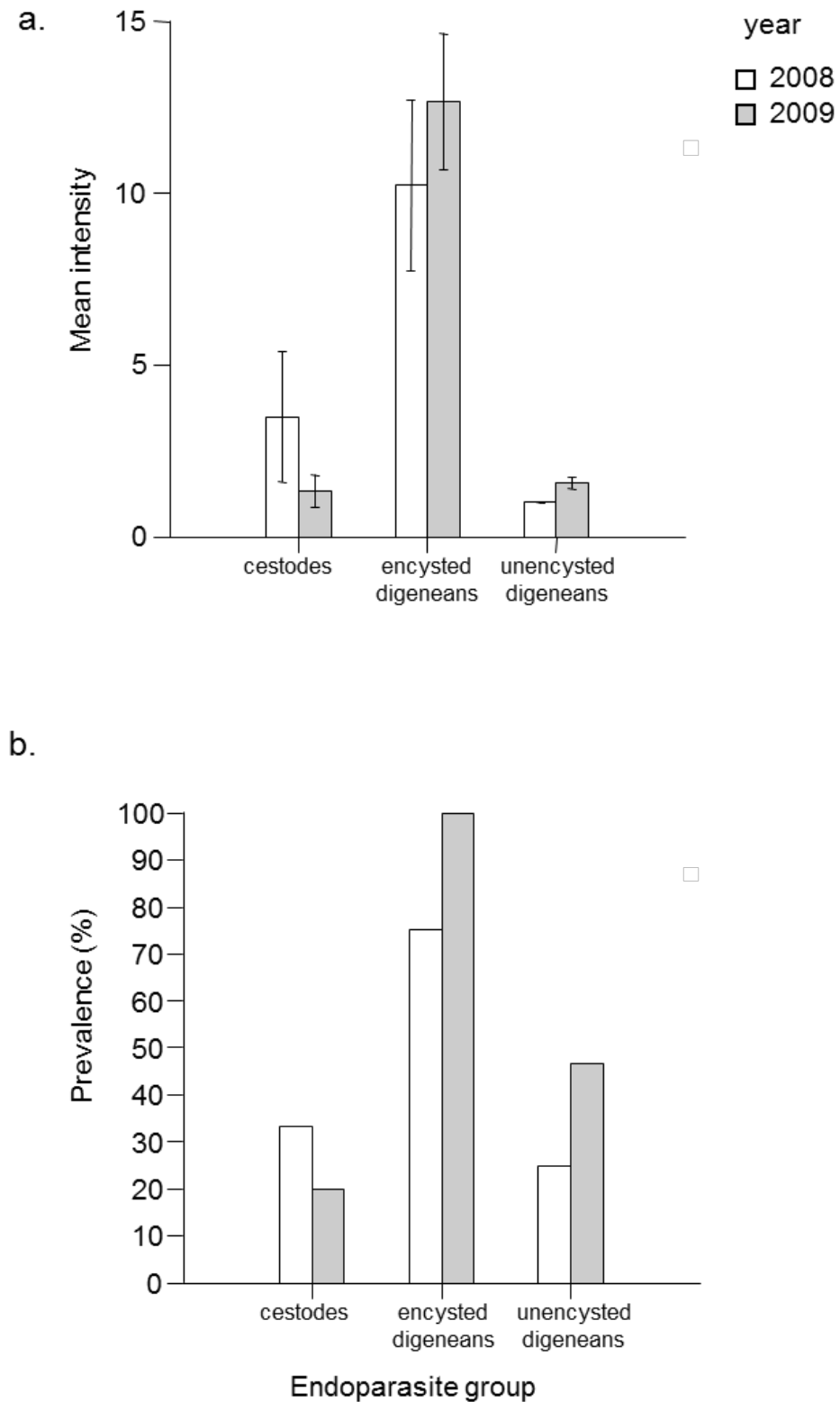


Figure 3-13 Comparison of (a) mean intensity and (b) prevalence of endoparasite groups from *Cassiopea* sp., between April 2008 and February 2009. For 2008, n = 12 jellyfish, for 2009, n=15 jellyfish

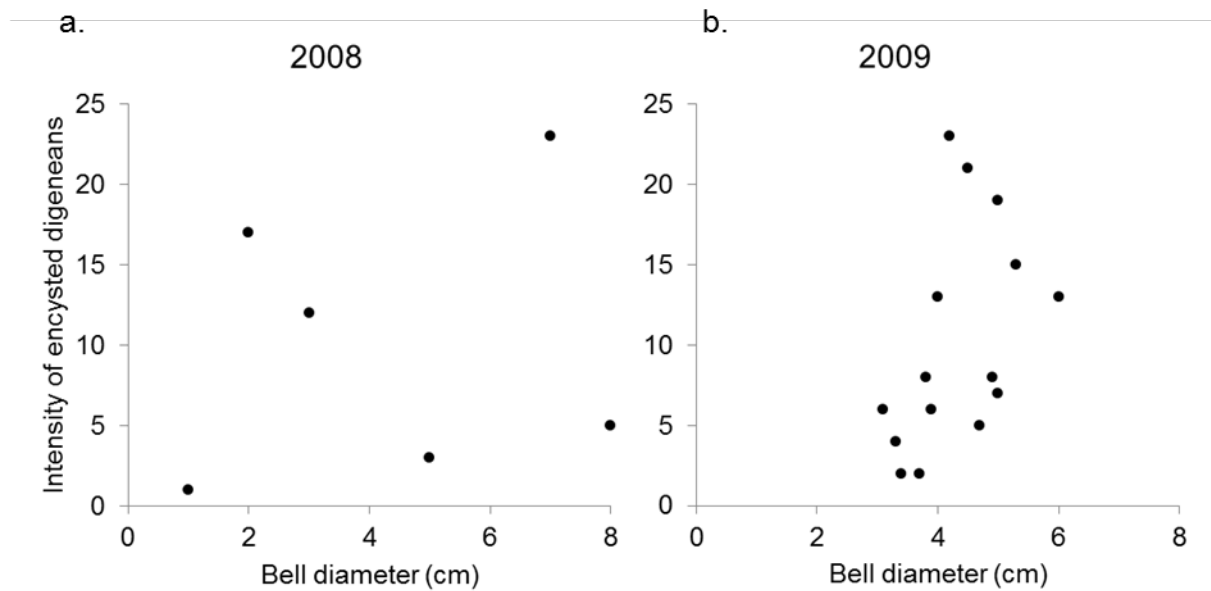


Figure 3-14 Intensity of encysted digeneans versus bell diameter of *Cassiopea* sp. in (a) 2008 and (b) 2009

3.3.5 Molecular Ecology

Sequences were obtained from 6 specimens of *Cassiopea* sp. digeneans. Sequences were categorised into 4 groups which were treated separately due to large sequence differences between groups. The first group allowed a tentative identification to species, the second group allowed a digenean to be tentatively placed into the Apocreadiidae, the third group was only able to be identified to class Trematoda, and the fourth group consisted of three identical sequences from three specimens confirming the presence of one species at two of the sampled locations (Lizard Island and Vlasoff Reef). The groupings and identifications are tentative because no replicate sequences were able to be obtained (other than for the fourth group), and the morphology of specimens was unable to be used for confirmation as characteristics necessary for identification were absent (e.g. reproductive organs), or unable to be identified in the metacercariae (due to the difficulty of excysting specimens, or the destruction of the single specimen in the DNA process) and because no information is available about the metacercarial stage of the identified adults.

One sequence obtained from a digenean from *Cassiopea* sp. at Lake Magellan was identical to a sequence obtained from the adult digenean, *Pseudopisthogonoporus vitellus* which had been obtained by Dr T. H. Cribb from the unicornfish *Naso vlaminghii* (family Acanthuridae) caught at Heron Island, on the southern

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GBR (23°27'S, 150°55'E). The two sequences were each 462 base pairs long. Only two individuals of this digenean morphotype were found in *Cassiopea* sp., both in the same jellyfish at Lake Magellan. The metacercariae were unencysted and speckled with red pigment (Figure 3-15). Two large pigment spots were present at one end of the metacercariae. The measured individual (Figure 3-15) was 100 µm long and 50 µm wide. No suckers could be distinguished.

A sequence was obtained for a specimen of Morphotype F from Crab Island (Figure 3-5). The initial sequence was 466 base pairs long and incorporated all of ITS2 (which was 306 bp long). After alignment with the nearest sequences obtained from Genbank, a distance matrix revealed 38 to 45 base pair differences (8.2-10%) to *Homalometron* species (family Apocreadiidae). The sequence was 63 base pairs different to another genus in the apocreadiid family *Schistorchis zancli*, and 94 and 95 base pairs different to the outgroup species in the neighbouring family Cryptogonimidae (*Varialvus charadrus* and *Siphoderina manilensis* respectively). Outgroups from the family Cryptogonimidae

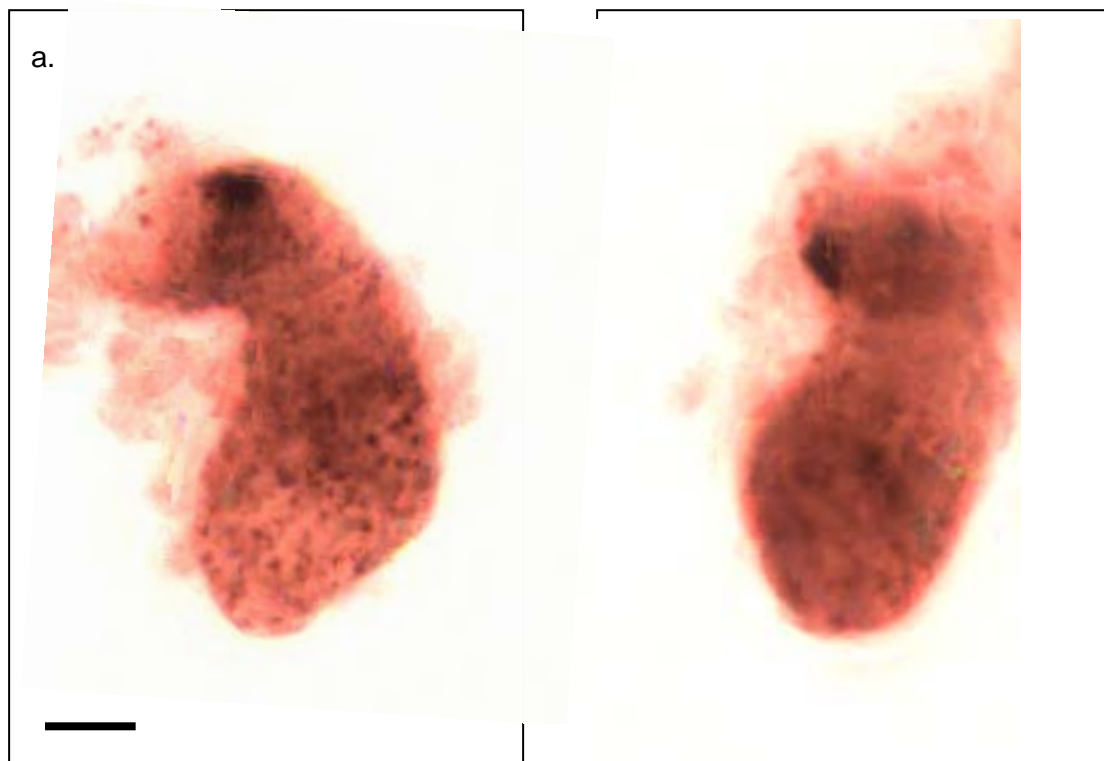


Figure 3-15 Metacercariae of *Pseudopisthoxoa*
lateral view. Scale bar represents 20 µm

ventral

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were chosen as it is the closest family related to the Apocreadiidae (Bray et al. 2009). Due to the limited number of apocreadiid ITS2 sequences available within Genbank, it is possible that the most closely related genus is an apocreadiid for which the sequence has not been obtained, or from another family entirely. Specimens of morphotype F were encysted in spherical cysts and had multiple pigment spots (Figure 3-5).

Three sequences from three digenean specimens of Morphotype A were obtained from *Cassiopea* sp. from Lizard Island and Vlasoff Reef. The three sequences ranged from 374 to 409 base pairs in length. When these were aligned and trimmed the fragments were identical. These fragments incorporated 251 base pairs of ITS2 (but not the entire ITS2). This confirms the presence of one digenean species at both locations. Sequences obtained from Genbank using the megablast (highly similar sequences) and blastn (somewhat similar sequences) had 45-51 (12.0-13.6%) base pair differences. These sequences were from the orders Echinostomida and Plagiorchiida. As the base pair differences were so great, and the closest groups from two different orders, further phylogenetic analysis is redundant until further sequences from identified digeneans are obtained. Morphotype A metacercariae were encysted and the cysts were ovoid in shape (Figure 3-5).

A sequence was obtained from one specimen of Morphotype I from *Cassiopea* sp. from Lizard Island. This sequence was 453 base pairs long, incorporating an incomplete ITS2 sequence of 283 base pairs. The closest sequences from Genbank and the authors' databases were 59-73 base pairs different. Similarly to Morphotype A, these sequences were from the orders Echinostomida and Plagiorchiida. Morphotype I was ovoid in shape with 3 pigment spots and prominent suckers (Figure 3-5).

3.4 Discussion

The four locations sampled supported a range of endoparasite morphotype richness and infection rates. The greatest number of morphotypes and intensity of endoparasites occurred at Lizard Island and, despite Lizard Island and Vlasoff Reef both being coral reefs located in the northern Great Barrier Reef, these locations shared only three morphotypes in common. The community composition of these two locations also differed significantly. Fewer morphotypes occurred at the two southern locations, with only two unique endoparasites types occurring at each location. Parasite populations are generally overdispersed, with the majority of hosts infected by few parasites, and a few hosts parasitised by the majority of the total parasite population (Anderson and May 1978; May and Anderson 1978). Therefore, to obtain accurate estimates of prevalence and intensity

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of infection of parasites in a host community high sample sizes are needed. It should be noted, therefore, that the infection parameters reported within this chapter are preliminary. Sample sizes were limited by the time consuming nature of jellyfish dissections and difficulty of identifying morphotypes.

Abundance and diversity of digeneans in definitive hosts are positively correlated with abundances and diversity in first intermediate hosts (Hechinger and Lafferty 2005). Also, the density (Thieltges and Reise 2007; Thieltges 2007) and distribution (Durieux et al. 2007; Durieux et al. 2010) of first intermediate hosts of digeneans affects digenean infection in second intermediate hosts. Therefore, locations that support high abundances and diversity of first intermediate and definitive hosts can be expected to have high diversity and abundances of digeneans in second intermediate hosts. This was consistent with the two coral reef locations on the GBR supporting the greatest number and abundances of parasite morphotypes. Moreover, Lizard Island has a higher diversity of habitats (including coral reef, lagoon, mangroves, seagrass and algae) than Vlasoff Reef, which may contribute to the higher diversity of potential hosts at Lizard Island. Gastropods, the primary first intermediate hosts of digeneans, are diverse and abundant at Lizard Island (Keable 1995). Diversity of definitive hosts is also likely to be greater at the GBR locations than the other locations, hence supporting a higher diversity of trematode species. This is supported by a study comparing the diversity of digeneans from 214 fish species on the Great Barrier Reef with that of 103 inshore fish species from Moreton Bay in southeast Queensland. Cribb et al. (1994) found 2.61 species of digenean per host on the GBR compared with 1.41 for the southern inshore group.

In contrast to the two coral reef locations, Lake Magellan and Crab Island were relatively isolated locations with minimal connection to adjoining habitats. Few fish and invertebrates (other than jellyfish) were observed in the isolated, hypersaline lagoon at Crab Island. Lake Magellan is a man-made lake at the centre of a housing development. The lake supports seagrass and some fish, including trevally, but it is separated from the sea by an artificial canal, with a pipe between the lake and the canal. The limited flow between the sea and both Crab Island and Lake Magellan likely restricts the movement of potential parasite hosts into these locations.

Some of the variability among locations may have occurred due to differences in the time at which each location was sampled. However, all four locations were sampled within two months during summer/autumn (January for Vlasoff Reef, February for Lizard Island and March for Crab Island and Lake Magellan). Consequently it is unlikely that the large spatial

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differences observed were confounded by seasonal changes in the endoparasite community. Moreover, the community of endoparasites sampled on two occasions (April 2008 and Feb 2009) did not differ, suggesting that the endoparasite community at Lizard Island, at least, is relatively stable. Few studies on temporal variation of jellyfish endoparasites exist, however, and this is recommended for future study, particularly in reference to life cycles of the endoparasites. Significant differences in prevalence (but not intensity) in infection of the hydromedusae, *Eirene tenuis* by the digenean *Opechona pyriforme* occurred between two sampling periods in October 1997 and July 1998 (Martell-Hernández et al. 2011). The hydromedusa was present in one other sampling period (May 1997) but was not parasitised by the digenean (Martell-Hernández et al. 2011). Two other studies investigating endoparasites of the ctenophore *Pleurobrachia pileus* sampled more intensively over a longer period and found seasonal variation in digenean metacercariae intensity and prevalence (Fraser 1970; Yip 1984). There were large changes in infection prevalences between some months but little change between others, the latter observation is consistent with the findings of the current study.

An interesting finding was that most morphotypes occurred at only one location and only two digenean morphotypes, one cestode morphotype, and one commensal copepod occurred at more than one location. Some digeneans which use jellyfish as hosts have extensive distributions. For example *Opechona bacillaris* is widely distributed in the southern and northern hemisphere, in both warm and cold temperate waters (Bray and Gibson 1990). In a study of the south-west Atlantic Ocean, all three digeneans identified to species level (*Monascus filiformis*, *Opechona* sp. and *Bacciger* sp.) were distributed along the coastline for a minimum of 20° of latitude (Diaz Briz et al. 2012). There have been too few studies of the spatial distribution of digeneans in jellyfish to be certain whether the limited distribution of most of the morphotypes in the current study is unusual. Many digeneans from fish, however, are known to have limited distributions. For example, in a survey of Pacific coral reef fish for sanguinicolid digeneans, which included similar locations to those used in this study, only one of the 19 digeneans was found at more than one location (Lizard Island and North Stradbroke Island) (Nolan and Cribb 2006). Similarly, Miller and Cribb (2007) found six of 13 species of *Retrovarium* occurred at only one location. In the current study, the shared morphotypes occurred at the two GBR sites, Vlasoff Reef and Lizard Island and may reflect their proximity, the sharing of a common habitat (both coral reefs) and the likely occurrence of similar first intermediate and definitive host species. Although Crab Island and Lake Magellan are located within 65 km

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of each other their habitats are very different and they are unlikely to share similar first intermediate and definitive hosts.

. The high salinity in the lagoon from which the *Cassiopea* sp. were collected at Crab Island may affect parasite presence and/or abundance. Salinity may affect both hosts and their parasites (e.g. Rogowski and Stockwell 2006). In the hydromedusan *Phialidium* sp., prevalence and maximum intensity of the digenean *Monascus filiformis* was lower near the mouth of the Río de la Plata than in adjacent sampled areas. The freshwater influence of the river was hypothesised to be the cause of this disparity (Girola et al. 1992).

Conversely, no correlation was found between salinity and prevalence and intensity of digenean *Opechona pyriforme* in *Eirene tenuis* in a hypersaline lagoon (Martell-Hernández et al. 2011).

Environmental variables may influence transmission and survival of endoparasites (Pietroock and Marcogliese 2003). Pollution can affect free living stages of parasites and their infective capabilities (review: Pietroock and Marcogliese 2003). With its close proximity to urban centres, Lake Magellan is likely to have higher pollution levels than the other locations. While ambient pollutant levels at all the collection sites are unavailable, concentrations of elements within *Cassiopea* sp. have been measured at Lake Magellan (Templeman and Kingsford 2010), Vlasoff Reef and Lizard Island (Templeman and Kingsford 2012). *Cassiopea* sp. bioaccumulate metals above ambient concentrations in their tissues (Templeman and Kingsford 2010; Templeman and Kingsford 2012). Although the data from the two studies are not directly comparable because bell and tissue concentrations were measured separately in urban environments, and together at GBR locations, there are patterns of higher element levels (e.g. copper, zinc, iron, manganese and aluminium) at Lake Magellan than the GBR locations (Templeman and Kingsford 2010; Templeman and Kingsford 2012; S. Templeman pers. comm.). These elevated concentrations of metals are indicative of anthropogenic pollution at Lake Magellan. Toxicants that affect transmission of endoparasites include copper, zinc, iron, manganese (digeneans, Cross et al. 2001), chromium (digenean, Wolmarans et al. 1988) and cadmium (digeneans, Holliman and Esham 1977; cestodes, Khalil et al. 2009). Therefore, the higher concentrations of metals at Lake Magellan may contribute to the low prevalences, intensities and morphotype diversity of endoparasites at this location.

This study is the first to record cestodes parasitising *Cassiopea* and the first to record a cestode parasitising a jellyfish in the Pacific Ocean. Cestodes were found in *Cassiopea* sp. at the two GBR locations, Lizard Island and Vlasoff Reef. There have been few previous

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studies of cestodes parasitising jellyfish and only one study has compared variation in infection parameters of scyphozoans between locations (Phillips and Levin 1973). No cestodes were observed in *Cassiopea xamachana* (n=4) or *C. frondosa* (n=3) in Florida but plerocercoid cestodes were recorded in *Stomolophus meleagris* in the Gulf of Mexico. Intensities of infection in the current study, however, were lower (one to three cestodes per jellyfish) than the maximum intensity of 10 plerocercoid cestodes recorded in *S. meleagris* (Phillips and Levin 1973). Prevalences of cestodes in the current study (20% at Lizard and 40% at Vlasoff Reef) were also lower than the 100% prevalence recorded in *S. meleagris* (n=500 jellyfish) in the Gulf of Mexico (Phillips and Levin 1973). The cestode parasites of jellyfish are poorly known, their definitive hosts are undetermined and none has been linked to identified adult species. The effect of the cestodes on jellyfish is unknown, although vacant burrows apparently left by the cestodes in the tissues of their hosts contain large populations of bacteria (Phillips and Levin 1973). Cestode larvae similar to those found in this study have been found in the fish *Caesio cuning* and *Plectropomus* at Lizard Island (Ian Beveridge pers. comm.).

The intensity of infection of digeneans at all sites was low, except for Morphotype A which was found at Vlasoff Reef and Lizard Island. Prevalences of total digeneans were higher at Lizard Island and Vlasoff Reef than at the non-GBR sites. Only two digenean morphotypes (Morphotype A and Morphotype H) occurred at multiple locations. Prevalence and mean intensity of Morphotype A were greater at Lizard Island than Vlasoff Reef. A review of digeneans in second intermediate crustaceans and bivalve hosts showed prevalence in host-parasite pairs often varied greatly between locations, while intensity and abundance often occurred within a narrow range (Thieltges et al. 2009a). Morphotype H was consistent with this pattern because although prevalences were slightly higher at Lizard Island than Vlasoff Reef, intensities were similar. Intensity is understood to be a characteristic of a host-parasite species pair, remaining similar between locations (Thieltges et al. 2009a). This pattern is particularly apparent for smaller hosts (e.g. amphipods), where their small size limits the maximum intensity, but not for larger hosts (e.g. bivalves). As *Cassiopea* sp. are relatively large animals, in which intensity should not be limited by host size intensity may be more variable between locations, as it was for Morphotype A. Prevalences of Morphotype A also varied greatly between Lizard Island and Vlasoff Reef. Prevalence in second intermediate hosts depends upon encounter rates with infective stages (e.g. digenean cercariae) which is subject to much variation on a local scale (Thieltges et al. 2009a). The abundance of infective stages relies upon the density, abundance and shedding rates of the first intermediate hosts. Local environmental

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variables such as temperature influence shedding rates (e.g. Koprivnikar and Poulin 2009) and first intermediate host populations may be influenced by the parasites themselves (Lauckner 1987). Transmission of infective stages may also be affected by predation by other fauna (e.g. *Opechona bacillaris* cercariae are preyed upon by hydroids, K  ie 1975). The variability of these many local factors contributes to the high variability of prevalence in second intermediate hosts between locations (Thieltges et al. 2009a).

Ten digenean morphotypes were rare and were represented by only one specimen. Their rare occurrence may be the result of accidental infections, whereby *Cassiopea* sp. are not the typical host. Due to the highly aggregated dispersal of parasites, too few jellyfish may have been sampled to reflect the true distribution of the morphotypes. Digenean populations may also vary temporally or the morphotypes may simply be rare. In the Atlantic Ocean, *Bacciger* sp., for example, parasitised only 0.1% of 17,163 *Liriope tetraphylla* hydromedusae compared with *Monascus filiformis* and *Opechona* sp. which parasitised 80.2% and 18.9% respectively (Diaz Briz et al. 2012). Until further information about the species present and their life cycles are known, answers to these questions are only speculative.

Cestodes and digeneans in *Cassiopea* sp. were primarily positioned in the oral arms, particularly in the distal ends and also the outer edges of the bell and oral disc. These regions may be more readily bitten by the definitive fish hosts (Ohtsuka et al. 2009) or they may be where the infective stages of the parasites first encounter the jellyfish. Cestode larvae recovered from three rhizostome jellyfish were also most common in the bell margins and oral arms (Moestafa and McConnaughey 1966).

DNA sequencing allowed the validation of several individuals as belonging to different morphotypes, confirmed the presence of Morphotype A at two locations, and the identification of one digenean to species. *Pseudopisthoganoporus vitellus* was found in a *Cassiopea* sp. from Lake Magellan. The unicornfish, *Naso vlaminghii*, hosted the adult digenean from which the identical sequence was obtained. Adult *P. vitellus* have also been recorded from the digestive tract of 5 other unicornfish, *Naso annulatus* (Bray and Justine 2012), *Naso brevirostris* (Pritchard 1963; Yamaguti 1970; Toman 1989; Machida and Uchida 1990; Bray and Cribb 1998; Justine et al. 2012), *Naso hexacanthus*, *Naso unicornis* (Pritchard 1963), *Naso lopezi* (Machida and Uchida 1990), and from the family Balistidae, the triggerfish *Melichthys vidua* (Pritchard 1963). The fish were from the Seychelles, Indian Ocean (Toman 1989); New Caledonia (Bray and Justine 2012); Ryukyu Islands, Japan (Machida and Uchida 1990); Heron Island, Australia (Bray and

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Cribb 1998); and Hawai'i (Pritchard 1963; Yamaguti 1970) indicating that this species of parasite is widely distributed. No records exist of any intermediate hosts, so the observation of *P. vitellus* in *Cassiopea* is a novel and important finding. The digenean is a member of the family Lepocreadiidae. The "typical lepecreadiid life cycle" (Cribb et al. 2003) was previously understood to involve a cercaria emerging from its first intermediate host (a prosobranch gastropod) and swimming to and penetrating a second intermediate host (an invertebrate or small vertebrate) in which it formed a metacercaria and waited for the host to be eaten by the definitive vertebrate host. More recent findings and phylogenetic analyses have suggested this life cycle is typical of some clades within the Lepocreadiidae, but that other life cycles also occur (Bray et al. 2009). Variations to the "typical" life cycle include cercariae using bivalves as a first intermediate host and encysting on the substrate (Hassanine 2006); and cercariae crawling to a second intermediate annelid host (Køie 1985). Thus, the presence of *P. vitellus* within *Cassiopea* sp. conforms to a 'typical' lepecreadiid life cycle. The distribution of *Naso vlaminghii*, *N. unicornis*, *N. annulatus* and *M. vidua* encompasses Lake Magellan (Kuitert 1993; OZCAM 2014) despite no existing records of the fish fauna in the lake. As adults, the *Naso* species are commonly reef-associated, however, juveniles are found in sheltered bays (Kuitert 1993). The species *N. annulatus*, *N. brevirostris* and *N. vlaminghii* have diets dominated by green filamentous algae and gelatinous zooplankton, while the closely related *Naso hexacanthus* feeds almost exclusively on large gelatinous zooplankton (Choat et al. 2002). This dietary information and the presence of metacercariae in *Cassiopea* sp. suggest that the digenean *P. vitellus* does indeed use jellyfish as an intermediate host to infect definitive fish hosts.

Despite extractions from 51 specimens, with numerous PCR runs for each extraction, there was a low rate of successful sequences. This may be partly due to the difficulty of obtaining DNA from such small specimens (all were <150 µm in length/diameter) and due to the preservation of samples. As digeneans could not be removed from live jellyfish, the process of preserving entire jellyfish specimens prior to removal of parasites may have caused some degradation of DNA. No PCRs using primers to extract *Isu*DNA (large sub unit DNA) were successful and this may be due to the length of the sequence (>1000 bp) which would be difficult to obtain with degraded DNA. In future analyses it is recommended that 18S or 28S genes are also attempted to be sequenced as these are from more highly conserved regions and would provide more information about which family the digeneans belonged to (Hillis and Davis 1988; Nolan and Cribb 2005).

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This study is the first to investigate endoparasites of a species of *Cassiopea*. It revealed differences in the communities of parasites infecting the jellyfish at two coral reef locations, and is the first study of spatial variation in jellyfish parasites to utilise DNA sequencing.

The large variability in parasite communities is hypothesised to be due to differences at the locations in a range of factors including host availability and diversity and habitat.

Cassiopea sp. was identified as a potential second intermediate host for the digenean *Pseudopisthomonoporus vitellus*. DNA sequences were obtained for a further three species of digenean parasites of *Cassiopea* sp., enabling the future identification of these species if the adults are sequenced.

4 Jellyfish host a diverse trematode fauna on the Great Barrier Reef, Australia

4.1 Introduction

Digeneans are a large and diverse group of trematodes with complex life histories, almost always involving multiple hosts (Cribb 2005). In the marine environment some digeneans have a simple two host life cycle, leaving their first intermediate host (usually a mollusc) and directly infecting their definitive host (generally a vertebrate). Others also use second intermediate hosts before infecting the definitive host, and some even have a third intermediate host. Second and third intermediate hosts may be invertebrates such as copepods or polychaetes, or vertebrates such as fish.

Jellyfish act as second intermediate hosts for at least 17 species of digeneans from six digenean families (Browne unpubl.). As there are approximately 70 families of digeneans in teleost fish alone (Cribb 2005), and the role of jellyfish as hosts has been understudied, it seems likely that there are more species which use jellyfish as hosts than is currently known. Jellyfish are important predators of zooplankton, fish larvae and other jellyfish (review: Mills 1995), are consumed by a diverse range of vertebrate predators (review: Arai 2005a), are widespread throughout the world's oceans (Lucas et al. 2014), and are often abundant (Pitt et al. 2014). These traits increase their potential to act as second intermediate hosts for digeneans, and they may be more important than presently understood.

Morphological identification of digenean metacercariae (the stage found in jellyfish hosts) is difficult as digenean taxonomy is based heavily upon reproductive organs of the adults. Some metacercariae share traits with sexual adults that enable their identification to family, genus or even species level. However, for many metacercariae, identification past family is not possible using morphology alone. Didymozoid metacercariae, for example, are not identifiable to species using only morphology (Anderson 1999). The matching of molecular sequences between adults and metacercariae is, therefore, an excellent identification tool (review: Nolan and Cribb 2005). In the marine environment, this technique has been used to match digenean species from first intermediate mollusc hosts to second intermediate crab hosts (e.g. Pina et al. 2007; Al-Kandari and Al-Bustan 2010; Al-Kandari et al. 2011); match metacercariae in fish second intermediate hosts, to adults in fish definitive hosts (Cribb et al. 1998; Anderson 1999); and to describe complete life

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cycles (e.g. with mollusc, shrimp and fish hosts, Jousson and Bartoli 2000). Even when sequences of identified adult digeneans are not available, DNA sequencing is useful for differentiating between species in larval stages (e.g. Anderson and Barker 1993), or determining the genus or family to which it may belong (e.g. Barnett et al. 2010). Internal Transcribed Spacer (ITS) rDNA sequences are well-suited to differentiating between digenean species as they are relatively well conserved within a species or genus (Nolan and Cribb 2005).

As DNA sequencing of jellyfish digeneans has not been used previously (apart from Chapters 2 and 3), morphologically similar metacercariae from jellyfish have not been previously differentiated on a molecular basis. The aims of this study were to examine the diversity, infection parameters and host specificity of digeneans parasitising three species of medusae and two species of ctenophores from the northern Great Barrier Reef, Australia, using a combination of molecular and morphological techniques; and to use molecular techniques to compare digeneans from jellyfish with those from other locations (e.g. Fitzroy Island and jellyfish from Chapters 2 and 3) and fish hosts.

4.2 Methods

4.2.1 Study Sites

Jellyfish were collected opportunistically from waters surrounding Lizard Island, (14°40'S 145°27'E) in the northern Great Barrier Reef (GBR) between the 10th and 25th of February 2009. Additional specimens were collected from Fitzroy Island (16°55'40"S, 145°59'14"E) on the 19th January 2009.

4.2.2 Jellyfish species

Three species of hydromedusae and two species of ctenophores were sampled from Lizard Island (Table 4-1). An additional hydromedusa and a ctenophore were collected from Fitzroy Island for use in molecular analyses. Jellyfish were captured by enclosing them within zip lock plastic bags whilst snorkelling. They were transported to the laboratory and retained alive in aerated seawater within the zip lock bags or buckets. Specimens were weighed and their bell diameters were measured. They were examined using an Olympus SZ61 stereo microscope and photographed with a Canon Powershot G6 digital camera and Canon software. The location of digeneans within the jellyfish was recorded and endoparasites were removed using forceps, dissection needles and a glass pipette. Digeneans were pipetted into near boiling vertebrate saline (0.85%; 1 part seawater: 3 parts freshwater) (Cribb and Bray 2010); placed on slides and examined using a Leitz

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Dialux 22 compound microscope and photographed with a Canon Powershot G9 attached to the microscope. Initially glass coverslips were used on the slides, enabling a 40x magnification to be employed. However, this resulted in the loss of small specimens upon removal of the coverslip, so concave slides without coverslips were subsequently used and photographed at a 25x magnification. Digenean specimens were preserved in 5% formalin. If replicate morphotypes were believed to occur, these replicates were preserved in 96% ethanol for subsequent genetic analyses. Hydromedusae were preserved in 5% borax-buffered formalin and representatives of each species preserved in 96% ethanol. Many species of ctenophore are very difficult to preserve. For example, members belonging to the genus *Bolinopsis* (class Tentaculata: order Lobata) normally completely disintegrate (e.g. Costello and Coverdale 1998; Gershwin et al. 2010). Thus only a photographic record of ctenophore specimens was retained.

4.2.3 *Morphological description of specimens*

Digenean specimens in formalin were stained, dehydrated, cleared and mounted onto slides in Canada balsam (see Chapter Two). The slides were viewed using an Olympus BX50 compound microscope, photographs were taken with a QImaging Go-21 CMOS camera mounted on the microscope and measurements were made using an ocular micrometer. The following morphological characters were measured: length and width, length of forebody, oral sucker width, oral sucker length, ventral sucker width and ventral sucker length. Forebody refers to the distance between the anterior extremity of the body and the posterior margin of the ventral sucker. Sucker width ratio is given with oral sucker as one. Body width:oral sucker width ratio is given with body width as one.

4.2.4 *DNA analysis*

Digeneans preserved in ethanol were used to extract DNA (see Chapter Two). The rDNA was sequenced for Internal Transcribed Spacer 2 (ITS2; see Chapter Two for methods) and the 5' end of the 18S rDNA gene. Amplification of the partial LSU rDNA region was performed using the primers LSU5 (5'-TAGGTCGACCCGCTGAAYTTAAGCA-3'; Littlewood et al. 2000) and 1500R (5' GCTATCCTGAGGGAACTTCG-3'; Tkach et al. 2003). PCR reactions for LSU rDNA were undertaken in a total volume of 20 µl, containing 3 µl of water, 10 µl of GoTaq, 1 µl of each primer, and 5 µl of template rDNA extract. The thermocycling profile was: 5 min denaturation hold at 96 °C; 37 cycles of 30 sec at 95 °C, 30 sec at 48 °C, 45 sec at 72 °C; a 5 min extension hold at 72 °C, then 1 min at 15 °C. Many unsuccessful attempts were made before obtaining only a single LSU sequence,

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and fungal contamination was an issue. The final successful sequence was obtained by cutting a gel band. Adult digenean specimens from the collection of Dr T.H. Cribb (University of Queensland, Australia) were also sequenced for ITS2 (Table 4-2). These specimens were selected from fish hosts suspected to be jellyfish predators.

4.2.5 Phylogenetic analyses

Sequence data were subjected to BLAST searches and also compared with sequence data from Dr T. H. Cribb's unpublished database. Sequences were selected from BLAST only if the Expectation (E) value was $<10^{-3}$ and query coverage was $\geq 60\%$. For the didymozoid analysis, sequences were obtained by searching "ITS2/Internal Transcribed Spacer 2" and "Didymozoidae". *Lecithaster stellatus* (family Lecithasteridae) was used as an outgroup. For the initial analysis of the Large Sub Unit (LSU) sequence, the closest 250 sequences (all with an E value of 0.0 and query coverage of 93-100%) were downloaded and then duplicate sequences/species removed. For the secondary LSU analysis, sequences of the families Atractotrematidae, Haploporidae, Troglotremitidae and Paragonomidae (the latter used as an outgroup) were downloaded. Outgroups for all trees were selected using Olson et al. (2003) except for the ITS2 Lepocreadioid tree.

Lepocreadioid ITS2 sequences were obtained from Dr T. H. Cribb's database, Genbank and from Dr Leonie Barnett (Central Queensland University). For all analyses, sequences were aligned on the CIPRES Science Gateway portal (Miller et al. 2010) using MUSCLE (Edgar 2004) with the defaults selected except the output option was changed to FASTA. Alignment was checked by eye in Mesquite (Maddison and Maddison 2011) and the ends were trimmed to match the shortest sequence. Distance matrices were constructed using MEGA 6.06 (Tamura et al. 2013) to calculate the number of base differences per sequence. Pairwise deletion was selected to remove ambiguous positions. The resulting matrix was used to remove duplicate sequences. Bayesian inference analysis was run on the CIPRES portal using MrBayes 3.2.1 on XSEDE (Ronquist and Huelsenbeck 2003). The best substitution model for the relevant data set was determined using jModeltest 2.1.1 (Guindon and Gascuel 2003; Darriba et al. 2012). The predicted models used were TVM+G for the didymozoid tree as predicted by the Akaike Information Criterion (AIC); GTR+I+G model for the Atractotrematidae LSU tree (AIC and Bayesian Information Criterion (BIC)); K80+G for the attractotrematid ITS2 tree (BIC); and TVM+I+G for the lepocreadioid ITS2 tree (AIC and BIC). Bayesian Inference Analysis was run over 10,000,000 generations with a sample frequency of 1000, with two runs each containing four simultaneous Markov Chain Monte Carlo (MCMC) chains. Default settings were used

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Table 4-1 Location, taxonomic information, size range and number of specimens collected for each jellyfish species. LI = Lizard Island, FI = Fitzroy Island

Location	Phylum	Order	Species	Bell diameter /body length (mm)	No. of specimens
LI	Cnidaria	Leptothecata	Aequorea australis	15-35	16
LI	Cnidaria	Leptothecata	Malagazzia carolinae	7-17	15
LI	Cnidaria	Limnomedusae	Olindias singularis	13-47	17
LI	Ctenophora	Lobata	Bolinopsis sp.	25-90	16
LI	Ctenophora	Beroidea	Beroe sp.	20-35	4
FI	Cnidaria	Leptothecata	Aequorea australis	45	1
FI	Ctenophora	Beroidea	Beroe sp. B	40	1

Table 4-2 Host fish locations and species of digeneans sequenced from Dr T. H. Cribb's collection. LI = Lizard Island, HI = Heron Island, ND = no data

Fish family	Fish species	Digenean family	Digenean species	Location
Acanthuridae	Naso vlaminghii	Lepocreadiidae	Pseudopisthgonoporus vitellus	HI
Balistidae	Abalistes stellatus	Lepocreadiidae	Hypocreadium	HI
Carangidae	Trachinotus blochii	Aepnidiogenidae	Neolepocreadium caballeroi	LI
Carangidae	Trachinotus bailloni	Lepocreadiidae	Clavogalea trachinoti	HI
Ostraciidae	Ostracion cubicus	Lepocreadiidae	Diploproctodaeum momoafata	LI
Pomacentridae	Acanthochromis polyacanthus	Lepocreadiidae	Lepotrema	ND

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except for the following (nst =6, rates=gamma, sumt burnin=3000, type of consensus tree = all compatible groups, sump burnin=3000). Tracer v1.6.0 (Rambaut and Drummond 2013) was used to confirm that log likelihood scores plateaued before 300,000 replicates, indicating that the appropriate burnin values had been chosen.

In addition to the Bayesian analysis, a maximum likelihood analysis and a neighbour joining analysis was run for each data set, to check whether there was consensus between the methodologies. Files were converted to Phylip format in Mesquite then uploaded to the RAxML Blackbox web interface (Stamatakis et al. 2008). The gamma model of rate heterogeneity was selected if appropriate, the 'maximum likelihood search' and 'estimate proportion of invariable sites' were also selected. A neighbour joining analysis was run for each data set within Mega 6.06 using 1000 bootstrap replications, maximum composite likelihood model, transitions and transversions substitutions, uniform rates between sites and pairwise deletion.

4.2.6 *Statistical analyses*

Infection parameters (prevalence, mean intensity, and range) were calculated according to Bush et al. (1997) (see Glossary). It is recognised that these parameters are preliminary, and unlikely to accurately represent the parasite population within the host species (see Section 3.4). Intensity and prevalence of total digeneans include all those originally counted from jellyfish (90 digeneans). This number is higher than those categorised into morphotypes (65 digeneans), as some were lost before they could be photographed due to the difficulty of successfully extracting live digeneans (often <200 μm) from jellyfish, straightening them, and transferring them to a slide.

Cluster analysis was used in PRIMER 6 to group morphotypes using morphological measurements. Ratios of body width/body length, forebody/body length, ventral sucker width/oral sucker width, and oral sucker width/body width were used rather than absolute measurements so that intraspecific size differences would not confound results. Values were square root transformed, a resemblance matrix using Euclidean distances was created, and this was used to construct a dendrogram. As all measurements are necessary to create a resemblance matrix, six morphotypes were unable to be utilised owing to missing measurements (e.g. suckers were sometimes unable to be measured).

4.3 Results

4.3.1 Overview

All five species of jellyfish sampled were infected by digeneans and the overall prevalence of infection was 34% (Table 4-3). On the basis of morphology, two superfamilies of digeneans, Lepocreadioidea (including family Lepocreadiidae) and Hemiuroidea (including families Hemiuridae and Didymozoidae) were recognised. On the basis of molecular analysis a third superfamily, Haploporoidea (family Atractotrematidae), was detected among specimens identified initially as Lepocreadioidea on the basis of their morphology. Not all morphotypes were sequenced (due to insufficient specimens or unsuccessful PCR attempts) and morphological data were limited for some sequenced specimens. Taken together the combined morphological and molecular data suggest the presence of 16 digenean species in these five species of jellyfish (Figure 4-1). Several of the digenean species showed evidence of distinct host and within host site specificity.

4.3.2 Morphological Diversity

Three superfamilies consisting of four families of digeneans were recognised. Didymozoid metacercariae were immediately recognisable because their gut consisted of a series of connected chambers (Anderson 1999). Other (non-didymozoid) hemiuroids were recognised by the loop in the forebody formed by the excretory vesicle. All other forms were initially identified as lepocreadioids because of the presence of a pair of suckers, an I-shaped excretory vesicle (when visible), spiny tegument, and extensive pigmentation in the forebody. Molecular analysis (Section 4.3.5) revealed that two forms initially identified as lepocreadioids were in fact attractotrematids. Although these forms were then recognisable, their morphology did not differ fundamentally from that of the lepocreadioids.

4.3.2.1 *Didymozoidae*

Didymozoid metacercariae were initially divided into two clear morphotypes based on morphology alone. The distinguishing characteristic was the smaller body length to width ratio of one morphotype (DBo2, 2.1) relative to that of the other morphotype (2.5-4.1) (Figure 4-1, Table 4-4). Morphotype DBo2 consisted of only one specimen. The remaining specimens were categorised according to their host jellyfish (Morphotype DBe from *Beroe* sp. and DBo from *Bolinopsis* sp.). Molecular analysis of DBo and DBe specimens of Morphotype DB revealed that it consisted of two species differing by 14 base pairs (bp) of ITS2 rDNA. Even with the molecular evidence, however, specimens of Morphotype DBo

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Table 4-3 Mean and range of intensity, prevalence (P), and n of p (i.e. number of infected hosts) for each digenean group/morphotype for each host jellyfish species. (Type B and C were combined as they were difficult to distinguish morphologically)

Taxon	<i>Aequorea australis</i>			<i>Malagazzia carolinae</i>			<i>Olindias singularis</i>			<i>Beroe</i> sp.			<i>Bolinopsis</i> sp.		
	n = 16			n=15			n = 17			n = 4			n = 16		
	Mean Intensity (range)	P (%)	n of p	Mean Intensity (range)	P (%)	n of p	Mean Intensity (range)	P (%)	n of P	Mean Intensity (range)	P (%)	n of P	Mean Intensity (range)	P (%)	n of P
SUPERFAMILY HAPLOPOROIDEA															
Atractotrematidae															
Type AA / AC combined	3.25 (1-7)	25	4												
SUPERFAMILY HEMIUROIDEA															
Type HA													1 (1)	6.3	1
Didymozoidae															
didymozoid										3.5 (2-5)	50	2	2 (1-3)	25	4
Hemiuridae															
<i>Lecithocladium</i> sp.							1 (1)	5.9	1						
SUPERFAMILY LEPOCREADIOIDEA															
Type LA							1.5 (1-2)	11.8	2						
Type LB	1 (1)	6.3	1												
Type LC	1 (1)	12.5	2										1 (1)	6.3	1
Type LD	2 (2)	6.3	1												
Type LE										2 (2)	25	1			
Type LF							1 (1)	5.9	1						
Type LG							2 (2)	5.9	1						
Type LH													1 (1)	6.3	1
Type LI										1 (1)	25	1			
Indeterminate	1.33 (1-2)	18.8	3	1 (1)	6.6	1	1 (1)	5.9	1						
Lepocreadiidae															
<i>Opechona bacillaris</i>	1 (1)	12.5	2							1 (1)	25	1			

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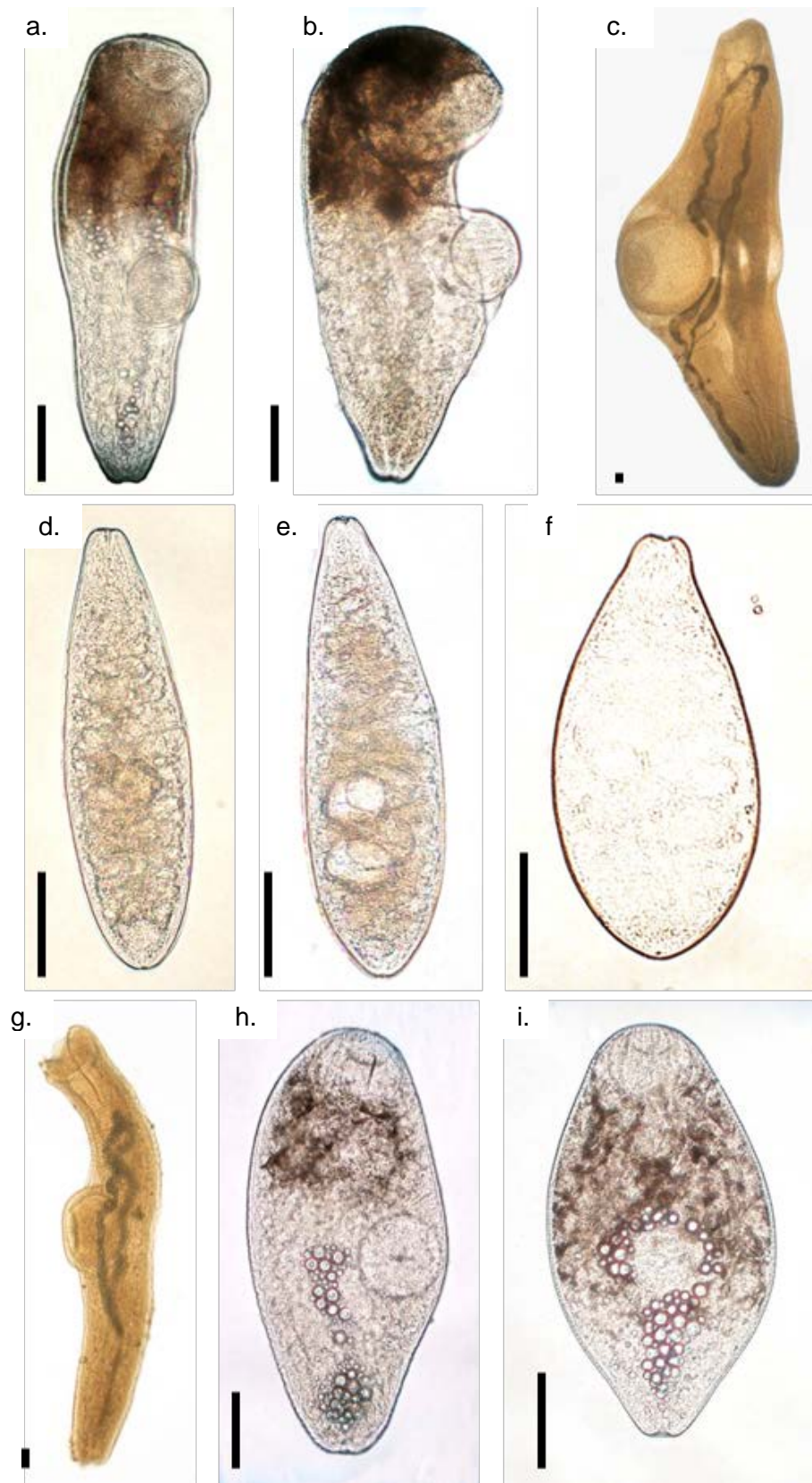


Figure 4-1 (a) Type AA (b) Type AB (c) Type HA (d) DBe (e) DBo (f) DBo2 (g) *Lecithocladium* sp. (h) Type LA (i) Type LB. Scale bars represent 50 µm, *cont. next page*

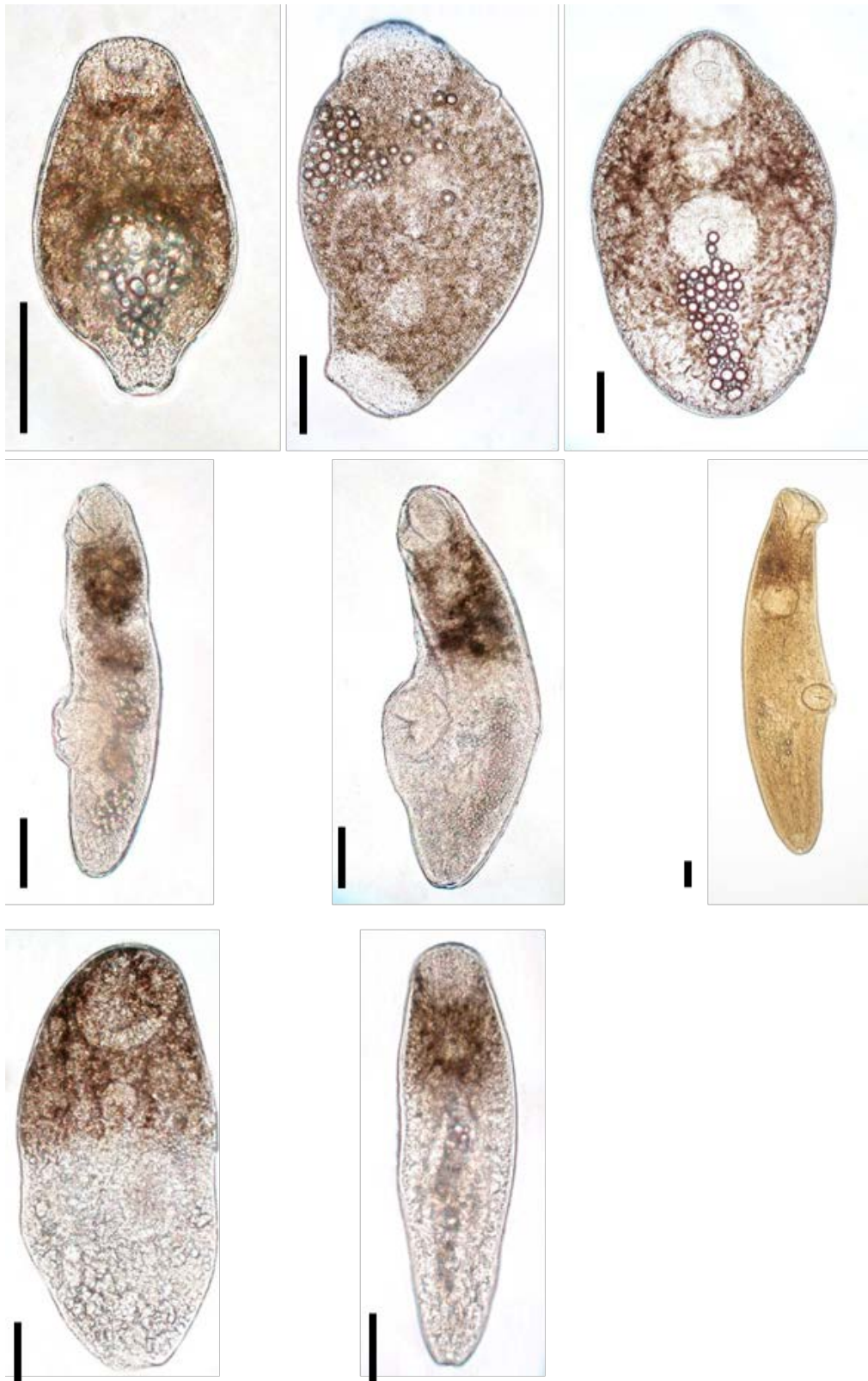


Figure 4-1 *cont.* (j) Type LC (k) Type LD (l) Type LE (m) Type LF (n) Type LG (o) Type LH (p) Type LI (q) *Opechona bacillaris*. Scale bars represent 50 µm.

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Table 4-4 Measurements of digeneans found in jellyfish hosts. Measurements are in micrometres and given as range, mean \pm standard deviation, and number of specimens for which that measurement was obtained.

Taxa (Host)	Length	Width	Forebody length	Oral sucker width	Oral sucker length	Ventral sucker width	Ventral sucker length	Length/width	Forebody as % of body length	Sucker width ratio	Body width: oral sucker width
SUPERFAMILY HAPLOPOROIDEA											
Family Atractotrematidae											
Type AA/AB (<i>ex-Aequorea australis</i>)	193-413	91.7-215	137-212	73-143	58.4-93	46.4-91.7	50.5-81.3	1.91-3.31	46.5-55.3	0.519-0.809	0.576-0.839
	339 \pm 19.2	142 \pm 12.4	180 \pm 8.43	96.5 \pm 6.94	75.7 \pm 3.44	62 \pm 3.91	64.4 \pm 2.44	2.73 \pm 0.159	50.8 \pm 0.788	0.65 \pm 0.0246	0.729 \pm 0.0289
	11	11	10	10	10	10	10	10	10	10	10
SUPERFAMILY HEMIUROIDEA											
Type HA (<i>ex-Bolinopsis</i> sp.)	2560	886	985	240	220	520	547	2.88	38.6	2.16	0.271
	2560	886	985	240	220	520	547	2.88	38.6	2.16	0.271
	1	1	1	1	1	1	1	1	1	1	1
Family Didymozoidae											
Type DBe (<i>ex-Beroe</i> sp.)	160-256	45.7-66	67.4-81.4	15.1-19.9	22.8-34.4	25.8-29.5	22.9-24.4	3.29-4.08	37.7-39.9	1.34-1.8	0.28-0.345
	196 \pm 11.6	53.5 \pm 2.6	73.5 \pm 4.15	17.4 \pm 0.808	27.8 \pm 2.45	27.3 \pm 1.13	23.5 \pm 0.448	3.66 \pm 0.114	38.5 \pm 0.718	1.62 \pm 0.141	0.319 \pm 0.0112
	7	7	3	6	4	3	3	7	3	3	6
Type DBo (<i>ex-Bolinopsis</i> sp.)	191-226	50.4-83.1	78.6-85.6	15.5-24.9	15.8-25.7	19.9-25.7	20.6-22.8	2.55-3.95	40.7-41.1	1.08-1.4	0.226-0.365
	209 \pm 5.04	68.2 \pm 3.8	82.1 \pm 3.5	19.1 \pm 1.25	21.5 \pm 1.67	22.8 \pm 2.9	21.7 \pm 1.1	3.12 \pm 0.175	40.9 \pm 0.233	1.24 \pm 0.161	0.282 \pm 0.0179
	7	7	2	7	6	2	2	7	2	2	7
Type DBo2 (<i>ex-Bolinopsis</i> sp.)	175	83.3	ND	20.4	20.7	ND	ND	2.1	ND	ND	0.245
	175	83.3		20.4	20.7			2.1			0.245
	1	1		1	1			1			1
Family Hemiuridae											
<i>Lecithocladium</i> sp. 966 (<i>ex-Olindias singularis</i>)		153	328	129	ND	ND	ND	ND	ND	ND	0.844
	966	153	328	129							0.844
	1	1	1	1							1
SUPERFAMILY LEPOCREADIOIDEA											
Type LA (<i>ex-O. singularis</i>)	265-508	136-290	113-254	56.8-80	53-86.1	56.1-104	57.4-103	1.75-2.17	42.8-50	0.926-1.3	0.276-0.439
	356 \pm 76.5	190 \pm 49.7	167 \pm 43.9	66.9 \pm 6.87	68.6 \pm 9.61	73.1 \pm 15.5	74 \pm 14.4	1.91 \pm 0.128	46 \pm 2.11	1.07 \pm 0.116	0.377 \pm 0.051
	3	3	3	3	3	3	3	3	3	3	3
Type LB (<i>ex-A. australis</i>)	217	119	101	40.8	37.2	38.8	35.4	1.83	46.7	0.951	0.344
	217	119	101	40.8	37.2	38.8	35.4	1.83	46.7	0.951	0.344

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Taxa (Host)	Length	Width	Forebody length	Oral sucker width	Oral sucker length	Ventral sucker width	Ventral sucker length	Length/width	Forebody as % of body length	Sucker width ratio	Body width: oral sucker width
	1	1	1	1	1	1	1	1	1	1	1
SUPERFAMILY LEPOCREADIOIDEA <i>cont.</i>											
Type LC	178	86.8	85	42.6	36.9	ND	ND	2.05	47.8	ND	0.491
(ex- <i>A. australis</i>)	178	86.8	85	42.6	36.9			2.05	47.8		0.491
	1	1	1	1	1			1	1		1
Type LD	234-263	143-150	ND	52.4	63.4	ND	ND	1.64-1.75	ND	ND	0.35
(ex- <i>A. australis</i>)	249±14.	146±3.4		52.4	63.4			1.7±0.059			0.35
	5	5						1			
	2	2		1	1			2			1
Type LE	273-316	164-196	105-135	59.2-60.4	55.7-72.9	58.5-69.9	57.1-63.7	1.61-1.66	38.5-42.6	0.988-1.16	0.308-0.361
(ex- <i>Beroe</i> sp.)	294±21.	180±15.	120±14.	59.8±0.6	64.3±8.6	64.2±5.7	60.4±3.3	1.64±0.02	40.6±2.04	1.07±0.08	0.334±0.0262
	5	9	8					48		46	
	2	2	2	2	2	2	2	2	2	2	2
Type LF	276	63.6	151	ND	44	ND	45.2	4.33	54.8	ND	ND
(ex- <i>O. singularis</i>)	276	63.6	151		44		45.2	4.33	54.8		
	1	1	1		1		1	1	1		
Type LG	338-356	105-122	129-175	45.4-59	54.8-61.3	54.7	59.4-73.1	2.78-3.4	36.1-51.8	1.2	0.373-0.562
(ex- <i>O. singularis</i>)	347±9.4	113±8.3	152±23.	52.2±6.8	58.1±3.25	54.7	66.3±6.85	3.09±0.31	43.9±7.83	1.2	0.468±0.0945
	2	2	2	2	2	1	2	2	2	1	2
Type LH	814	194	433	115	108	76.8	58	4.21	53.2	0.667	0.595
(ex- <i>Bolinopsis</i> sp.)	814	194	433	115	108	76.8	58	4.21	53.2	0.667	0.595
	1	1	1	1	1	1	1	1	1	1	1
Type LI	355	167	ND	78.8	82.4	ND	ND	2.13	ND	ND	0.473
(ex- <i>Beroe</i> sp.)	355	167		78.8	82.4			2.13			0.473
	1	1		1	1			1			1
Indeterminate	229	53.5	ND	ND	ND	ND	ND	4.28	ND	ND	ND
	229	53.5						4.28			
	1	1						1			
Family Lepocreadiidae											
<i>Opechona bacillaris</i>	259-315	68-83.8	141-169	37.2-47.3	33.8-45.4	30.5-38.1	36.4-39.4	3.11-4.63	53.8-54.7	0.719-0.941	0.447-0.624
(ex- <i>A. australis</i>)	288±16.	78.3±5.1	156±8.1	42.3±2.92	40.7±3.54	34.5 ±2.21	38.2±0.90	3.74±0.45	54.3±0.25	0.822±0.0645	0.545±0.0518
	3	7	6				6	8	8		
	3	3	3	3	3	3	3	3	3	3	3

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and DBe could not be distinguished morphologically or with a cluster analysis utilising morphological characteristics (Figure 4-2). Therefore, combined morphological and molecular analysis revealed at least 3 didymozoid species.

4.3.2.1 *Non-didymozoid Hemiuroidea*

Two individual digenean metacercariae were identified as belonging to the superfamily Hemiuroidea. Characteristics enabling their placement in this superfamily were the presence of an oral and ventral sucker, and the joining of the arms of the excretory vesicle to form a loop in the forebody. These two individuals were not sequenced as they were preserved in formalin as morphological specimens. They can be confidently distinguished as two species.

One individual was identified as *Lecithocladium* sp. (family Hemiuridae). The characteristics used for the identification were as follows: body surface with plications, well developed ecsoma, excretory arms united in forebody, funnel-shaped oral sucker, and elongated pharynx (Gibson 2002). As there are 83 nominal species of *Lecithocladium*, and the taxonomy of the genus is difficult, even with mature adult specimens (Bray and Cribb 2004) identification of this metacercariae to species was not undertaken.

The remaining non-didymozoid hemiuroid (Morphotype HA) was unable to be identified to genus. It was distinguished from *Lecithocladium* by the absence of plications, the lack of an ecsoma, the larger sucker length ratio and its much larger size (2560 µm compared with 966 µm for *Lecithocladium*). In the cluster analysis, it formed a clade with didymozoid Morphotype DBe, which was quite separate from other groups (Figure 4-2).

4.3.2.2 *Superfamily Haploporoidea*

Molecular analyses suggest that Morphotypes AA and AB belong to the family Atractotrematidae (superfamily Haploporoidea). Observed characteristics of these morphotypes that are traits of this family include: the spinous tegument; eyespot pigment being dispersed in the forebody; oral sucker terminal or slightly sub-terminal; intestine bifurcate, blind; excretory vesicle Y-shaped, pore terminal. The morphotypes were separated using molecular analysis (see section 4.3.5.2), however, as the specimens for Morphotype AB were damaged, no morphological characters were able to distinguish the pair and they were grouped for measurements and cluster analysis (Table 4-4).

Morphotypes AA and AB were both encysted. The cluster analysis (Figure 4-2) grouped

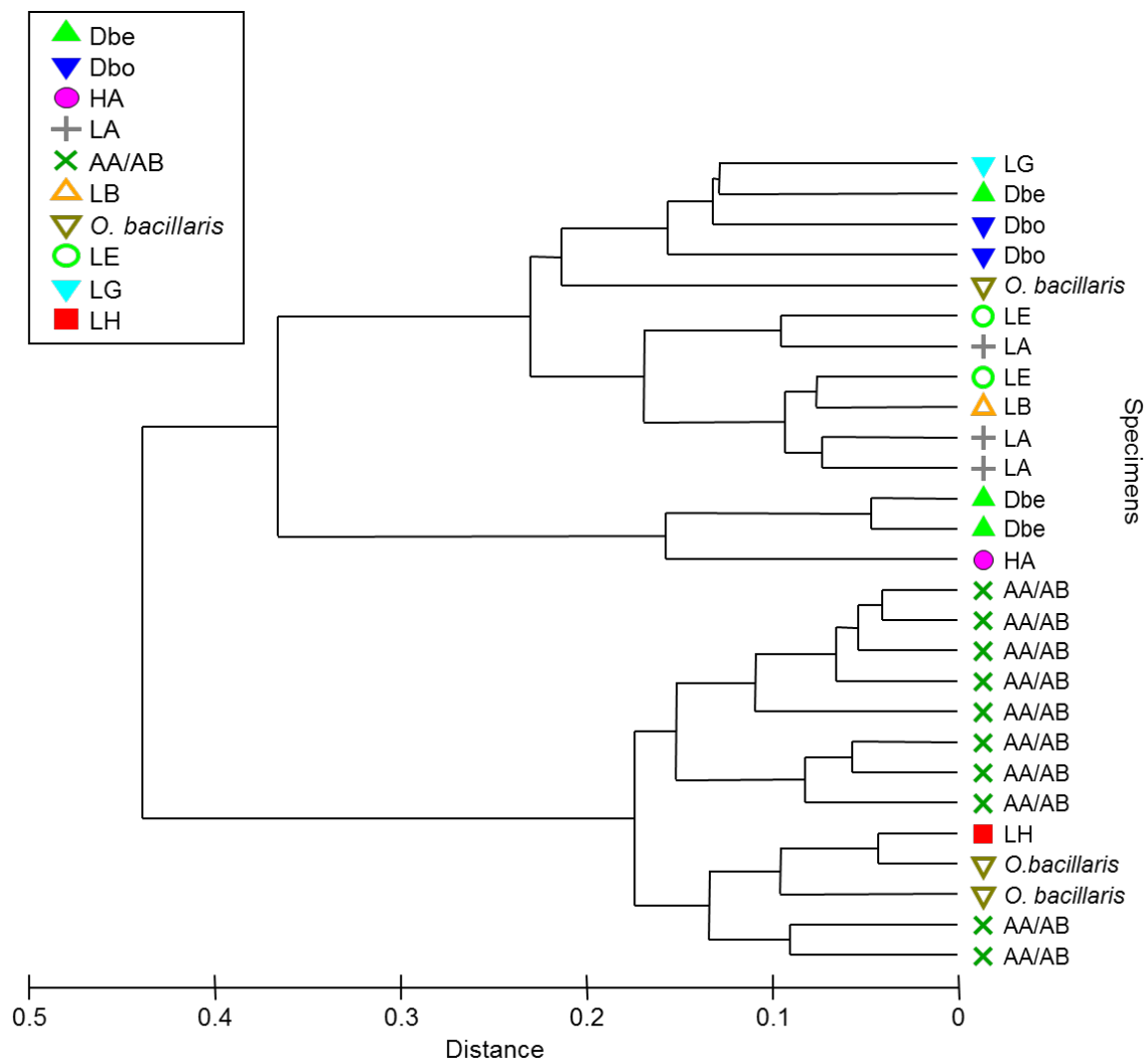


Figure 4-2 Dendrogram from cluster analysis of morphotypes based on measurement ratios. Each symbol represents the morphological measurements of an individual digenean. The degree of similarity between individuals is indicated by proximity. Ratio measurements (body width/body length; forebody/body length; ventral sucker width/oral sucker width; and oral sucker width/body width) were used

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eight specimens of the combined Morphotypes AA and AB, and two were grouped in a sister clade to *Opechona bacillaris* and Morphotype LH.

Of the four genera within the family Atractotrematidae Yamaguti 1939, Morphotype AA most closely resembles *Isorchis*. Characteristics which it shares with this genus include: the position of the ventral sucker in the midbody; body fusiform; usually more blunt anteriorly than posteriorly; oral sucker terminal (Overstreet and Curran 2005).

4.3.2.3 *Lepocreadioidea*

Ten morphotypes were identified as belonging to the superfamily Lepocreadioidea. Of these only one could be identified to species, *Opechona bacillaris* (family Lepocreadiidae). Six individuals with pigmentation similar to the other lepocreadioids were squashed or broken before identification and were categorised as indeterminate.

Three individuals were identified using the key of Bray and Gibson (1990) as *Opechona bacillaris*. The following characteristics were observed: Body elongate. Eye-spot pigment heavy in region from oral sucker to more than halfway to ventral sucker. Oral sucker large, aperture wide, slightly ventrally subterminal. Prepharynx distinct (the oesophagus and pseudoesophagus were obscured by pigment and could not be seen in these samples). Intestinal bifurcation in forebody. Forebody long (range: 53.8-54.7%, mean: 54.3%) of body length. Ventral sucker: rounded, smaller than oral sucker, sucker-width ratio 1: 0.72-0.94 (0.82), in posterior half of body, slightly protuberant (differs from adult which is in anterior third of body and distinctly smaller than oral sucker). Excretory pore terminal.

Whilst two *Opechona bacillaris* were grouped in the cluster analysis, the third specimen was separated. This specimen was slightly side on when measured. The remaining morphotypes were distinguished from each other using the following characteristics.

Morphotype LA was plump with the oral sucker smaller than the ventral sucker.

Morphotype LB was also plump, however the pigment extended past the ventral sucker and the oral sucker was cup-shaped. Morphotype LC was spindle-shaped and pigmented everywhere; the oral sucker was approximately equal in size to the ventral sucker.

Morphotype LD was fusiform and heavily pigmented throughout the body except at either end; the suckers were difficult to distinguish due to the heavy pigmentation. Morphotype LE was the only encysted lepocreadioid morphotype, specimens were oval, pigmentation extended past the oral sucker and the pharynx was large and distinct. Morphotype LF was cigar-shaped with a long forebody, and pigment extending halfway to the oral sucker.

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Morphotype LG was similar to Morphotype LF; however, the forebody of Morphotype LG was shorter and there was swelling surrounding the ventral sucker. Morphotype LH had an elongate body with a funnel-shaped oral sucker, and a small ventral sucker, pigment extended between the oral sucker and the pharynx. Morphotype LI was elongate-oval, the oral sucker was wide and pigmented, and the pigment extended almost to the base of the ventral sucker.

4.3.3 Infection parameters

Prevalence of infection by all digeneans (combined) varied greatly between the host species, ranging from 6.7% to 100% (Figure 4-3). It was highest in the ctenophore *Beroe* sp., but only four individual of that species were examined. Prevalence exceeded 40% for the ctenophore *Bolinopsis* sp. and the hydromedusan *Aequorea australis* (Figure 4-3). The lowest prevalence occurred in *Malagazzia carolinae* with only one of 15 individuals infected. For individual morphotypes, the highest prevalences were for the didymozoids in *Beroe* sp. (50%), followed by didymozoids in *Bolinopsis* sp., attractotrematid Types AA/AB combined in *A. australis*, and *Opechona bacillaris* and lepopocreadioids Type LE, and LI in *Beroe* sp. (all 25%).

The mean intensity of infection by all digeneans was highest in *Beroe* sp. (5.8), lowest in *Malagazzia carolinae* (1) and in a similar range for the other host jellyfish (3-4.4) (Figure 4-4). Didymozoids had a high intensity in the ctenophores (maximum of 5 in *Bolinopsis* sp., 3 in *Beroe* sp.), driving the high total intensity in *Beroe* sp. (Figure 4-4). The highest intensity of all morphotypes was the attractotrematids Type AA/AB in *Aequorea australis* (7). Other morphotypes had a low intensity of one or two digeneans per jellyfish (Table 4-3).

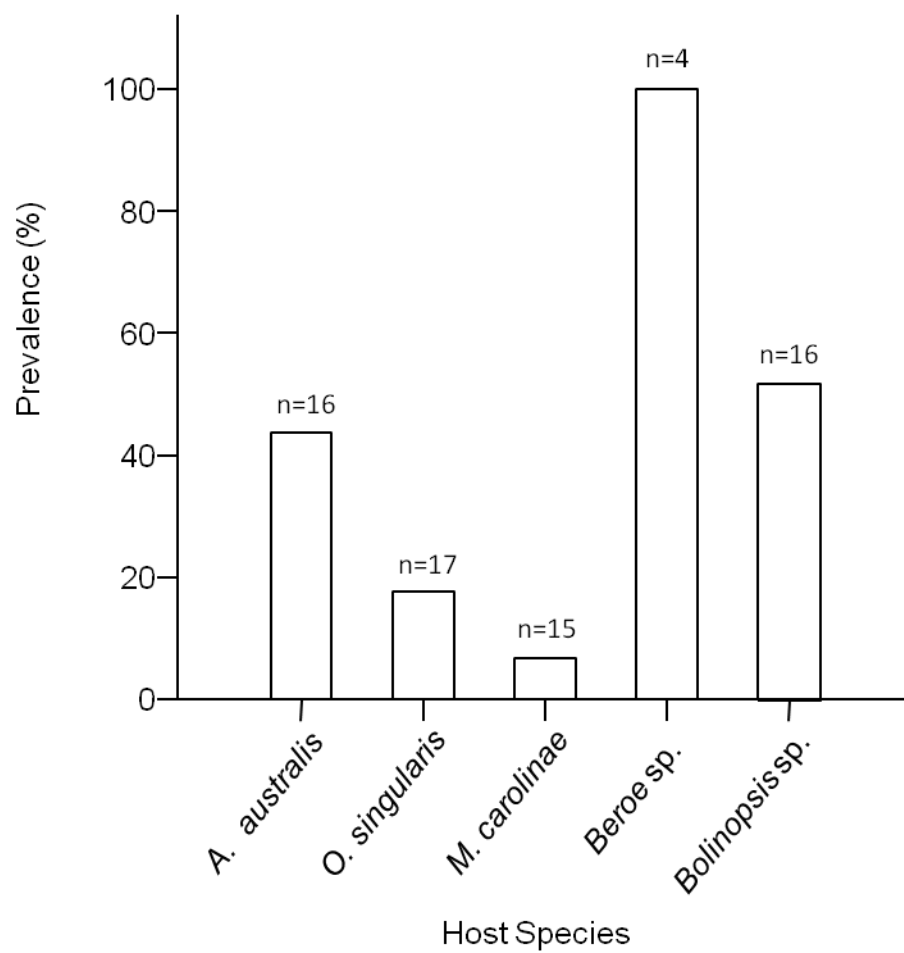


Figure 4-3 Prevalence of infection in host jellyfish species. n refers to the number of host individuals sampled per host species

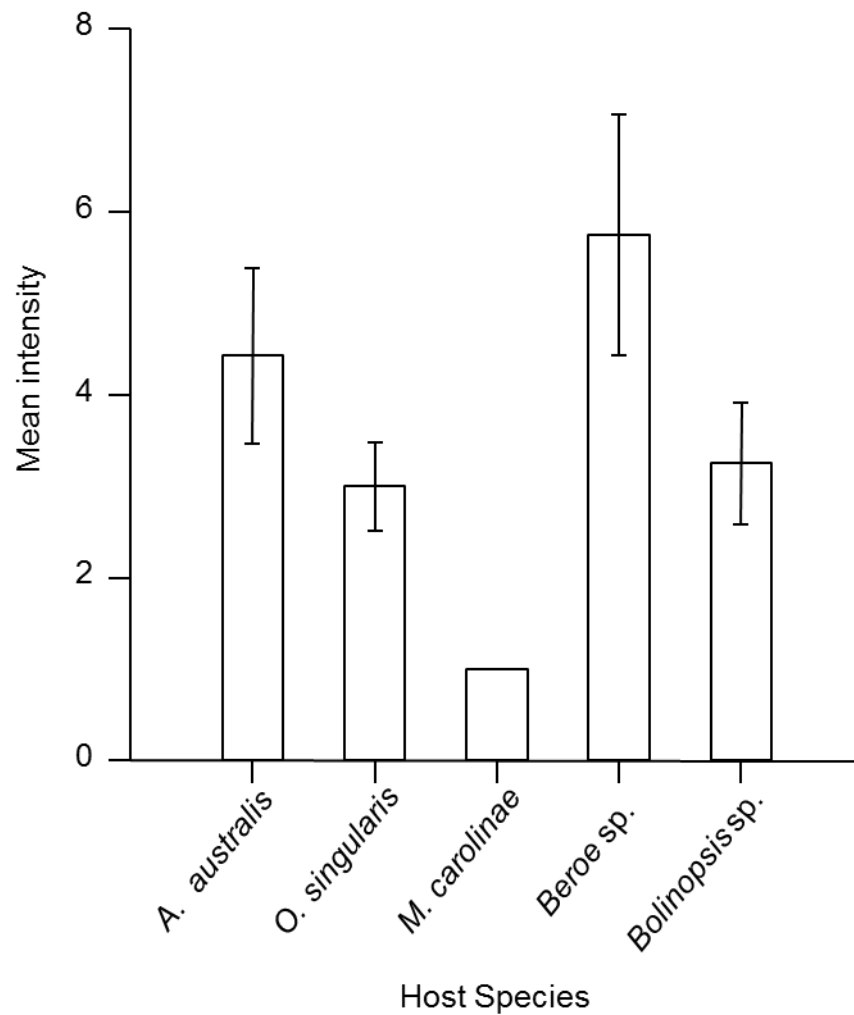


Figure 4-4 Mean intensity of infection of total digeneans for each host jellyfish species. Error bars indicate standard error

4.3.4 Location of digeneans within jellyfish

All digeneans were located within the mesoglea of the hydromedusae and ctenophore hosts with the exception of six individuals. Of these, three didymozoids (morphotype DBo) were located within the ctene rows of their *Bolinopsis sp.* host. One didymozoid (morphotype DBo) was situated in the pharynx of a *Bolinopsis sp.*. Both non-didymozoid hemiuroid individuals were located in the gastrovascular systems of their hosts, one in the radial canal of *Olindias singularis* (*Lecithocladium sp.*: Figure 4-5a) and the other in the pharynx of the ctenophore *Bolinopsis sp.* (Type HA, Figure 4-5b).

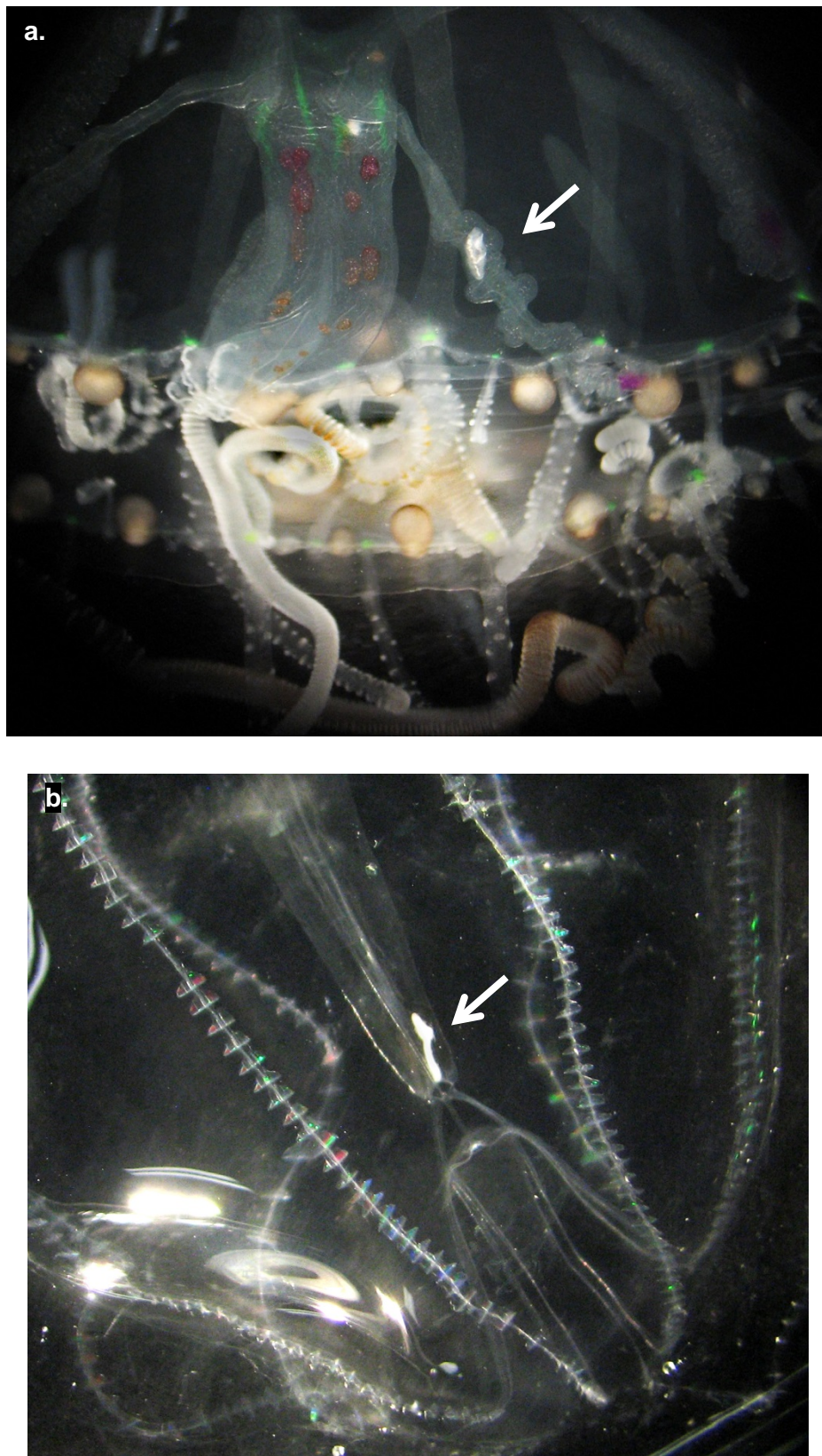


Figure 4-5 (a) *Lecithocladium* sp. in the radial canal of hydromedusan *Olindias singularis* (b) Hemiuroid digenean (indicated by arrow) in the pharynx of a *Bolinopsis* sp. ctenophore

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4.3.5 Molecular Relationships

4.3.5.1 Didymozoidae

The four sequences obtained from didymozoids hosted by ctenophores were each 517 nucleotides long. The sequences incorporated the complete ITS2 region (347 nucleotides), 123 bp of the 3' end of the 5.8S rDNA, and the beginning 47 nucleotides of the 5'S end of the 28S rDNA. The two didymozoid sequences from *Beroe* sp. (DBe) were identical, as were the two from *Bolinopsis* sp. didymozoid sequences (DBo) but the DBe and DBo morphotypes differed by 14 base substitutions. There were 30 didymozoid ITS2 sequences available on Genbank for comparison and most were relatively short. Alignment of these with the ctenophore didymozoids (with *Lecithaster stellatus* used as an outgroup) yielded only 205 characters for analysis. The didymozoids most closely related to those in ctenophores differed by 7-9 bp (3-4%) and belonged to the genus *Didymocystis*. Bayesian inference analysis of the dataset produced a phylogram (Figure 4-6) in which the didymozoids from ctenophores nested within the Didymozoidae. The didymozoids from *Beroe* sp. were sister to the didymozoids from *Bolinopsis* sp. in a clade that was sister to a clade containing species of *Didymocystis*, *Platocystis*, *Koellikerioides* and *Koellikeria*. This suggests that the didymozoids from ctenophores are more closely related to each other than the other sequenced didymozoids. The remaining taxa formed a clade separate to these, containing unidentified species of Didymozoidae, and species of *Helicodidymozoon*, *Indodidymozoon*, *Neometadidymozoon* and *Rhopalotrema* which are all parasites of platycephalids (flatheads).

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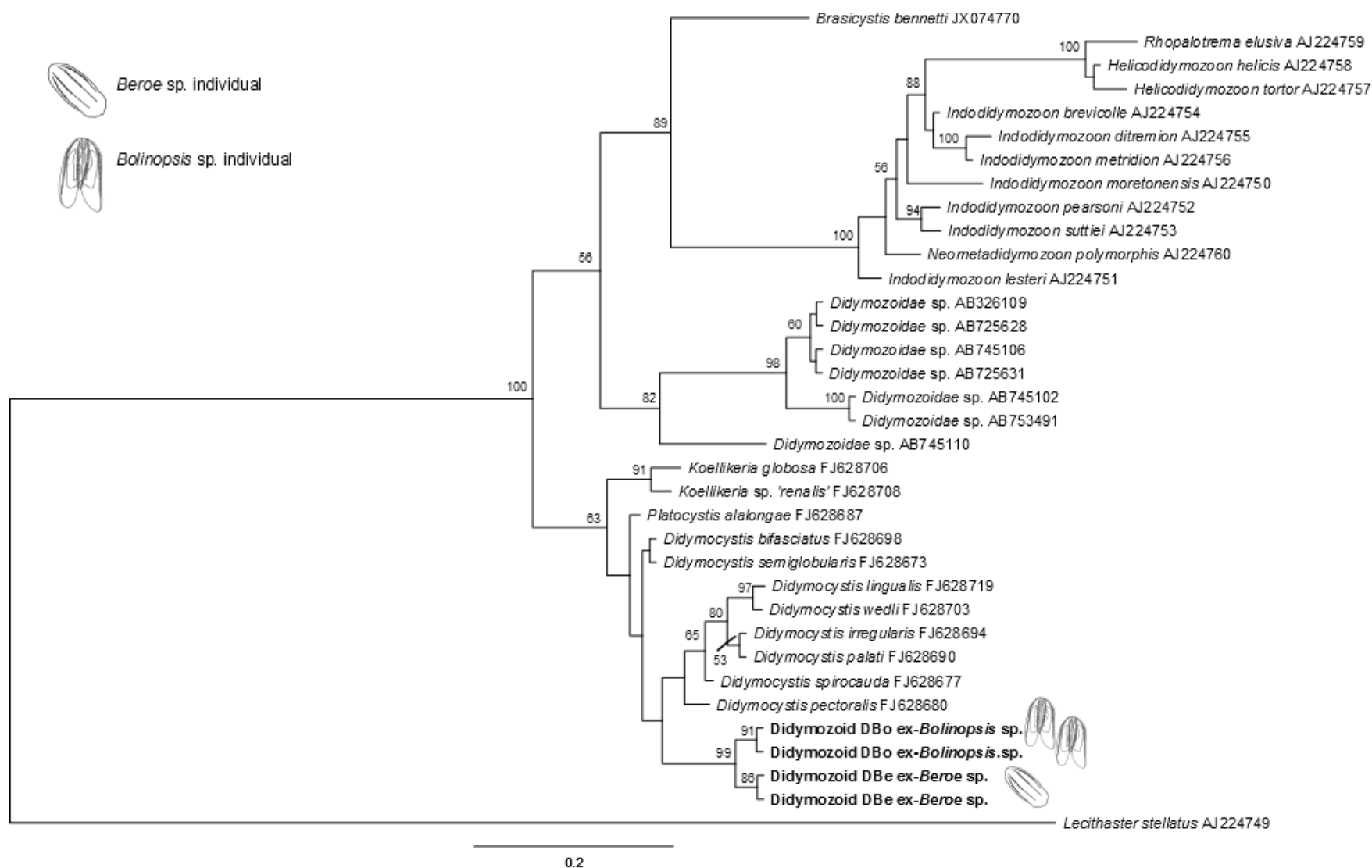


Figure 4-6 Relationships between Didymozoidae hosted by ctenophores (ex-*Bolinopsis* sp. and ex-*Beroe* sp., in bold) and other didymozoid taxa based on Bayesian inference analysis of the ITS2 rDNA dataset (employing a TVM + G substitution model). Posterior probabilities are at the nodes, values <50 are not shown. The scale bar indicates branch length (the expected number of substitutions per site)

4.3.5.2 *Atractotrematidae*

One partial LSU rDNA sequence was obtained from a single specimen (Morphotype AB) hosted by *Aequorea australis*. The raw sequence obtained was 1254 bp long. When aligned with 165 Genbank sequences, 1181 characters were used for analysis. A neighbour joining analysis (not shown) found a clear association of Morphotype AB with the family Atractotrematidae, and adjacent to the closely related families Haploporidae and Troglotrematidae (Jones 2005). Therefore a Bayesian inference analysis was undertaken with LSU sequences available from Genbank for the families Haploporidae, Atractotrematidae, Troglotrematidae and using a member of the Paragonomidae as an outgroup. The analysis of 24 sequences used 1148 bp. The analysis clearly groups the jellyfish digenean, Morphotype AB, within the Atractotrematidae, which were sister to the Haploporidae (Figure 4-7). The closest sequence was the attractotrematid *Pseudomegasolena ishigakiense* which differed from Morphotype AB by 35 bp (3%). The next closest sequence was also an attractotrematid, *Atractotrema sigani* (125 bp, 11%).

Four ITS2 sequences were obtained from four attractotrematids (Morphotypes AA and AB) hosted by two specimens of *Aequorea australis*. The sequences were 423 to 437 bp long. They incorporated the complete ITS2 region (253-269 nucleotides), 123 bp of the 3' end of the 5.8S rDNA, and the beginning 46 nucleotides of the 5'S end of the 28S rDNA. The four specimens produced two pairs of identical sequences that differed by only six bp when aligned (1% difference). A Bayesian analysis was undertaken with sequences from two attractotrematid adult specimens of *Pseudomegasolena ishigakiense* (hosted by *Scarus* species) from Genbank, and one sequence from an attractotrematid cercaria, *Cercaria queenslandae* II (putatively that of *Atractotrema sigani*) (Cannon 1978), from the gastropod (*Clypeomorus batillariaeformis*) from Dr T. H. Cribb's database. The two sequences of adult *P. ishigakiense* from two species of parrotfish were identical. A haploporid sequence from Genbank (*Haploporus benedeni*) was used as an outgroup. The analysis consisted of 366 characters. Two of the sequences from *A. australis* (Morphotype AB) formed a clade with the cercaria sequence (differing by seven bp) (Figure 4-8). These formed a sister clade to the remaining two sequences from *A. australis* digeneans (Morphotype AA) and differed by six bp. These in turn formed a sister clade to the *P. ishigakiense* from the parrot fish (differing by 20 to 21 bp). The analysis suggests the digeneans from *A. australis* are more closely related to each other than other attractotrematids, and are more closely related to the putative cercaria than to *P. ishigakiense*. Combining all molecular and morphological evidence, Morphotype AA is

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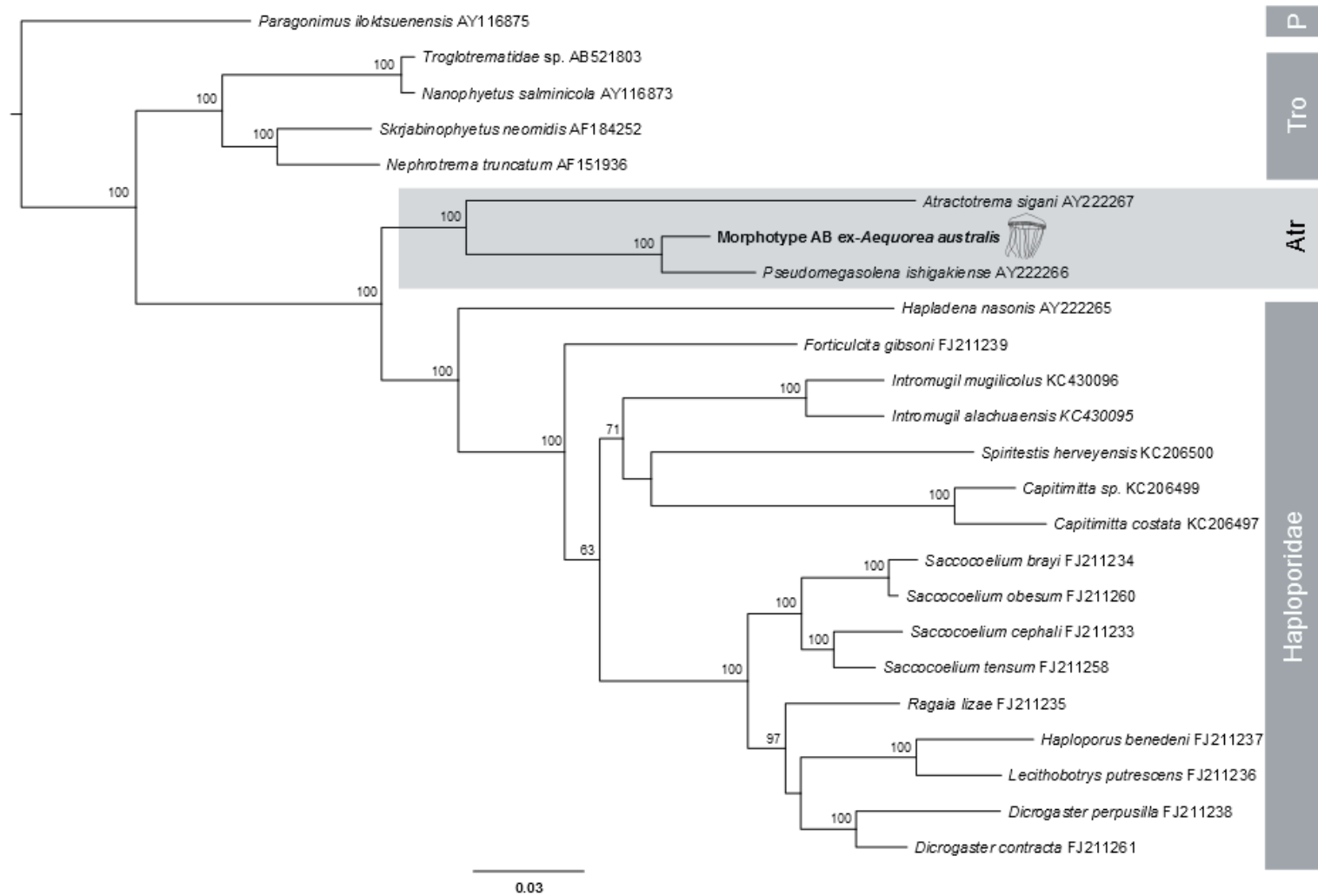


Figure 4-7 Relationships between Atractotrematidae hosted by hydromedusan *Aequorea australis* (bold) and other taxa from the superfamily Haploporoidea based on Bayesian inference analysis of the LSU rDNA dataset (employing a GTR+I+G substitution model). Posterior probabilities are at the nodes, values <50 are not shown. The scale bar indicates branch length (the expected number of substitutions per site). Families are based on (Blair et al. 2008). *Abbreviations*: Atr, Atractotrematidae; Tro, Troglotrematidae; P, Paragonomidae

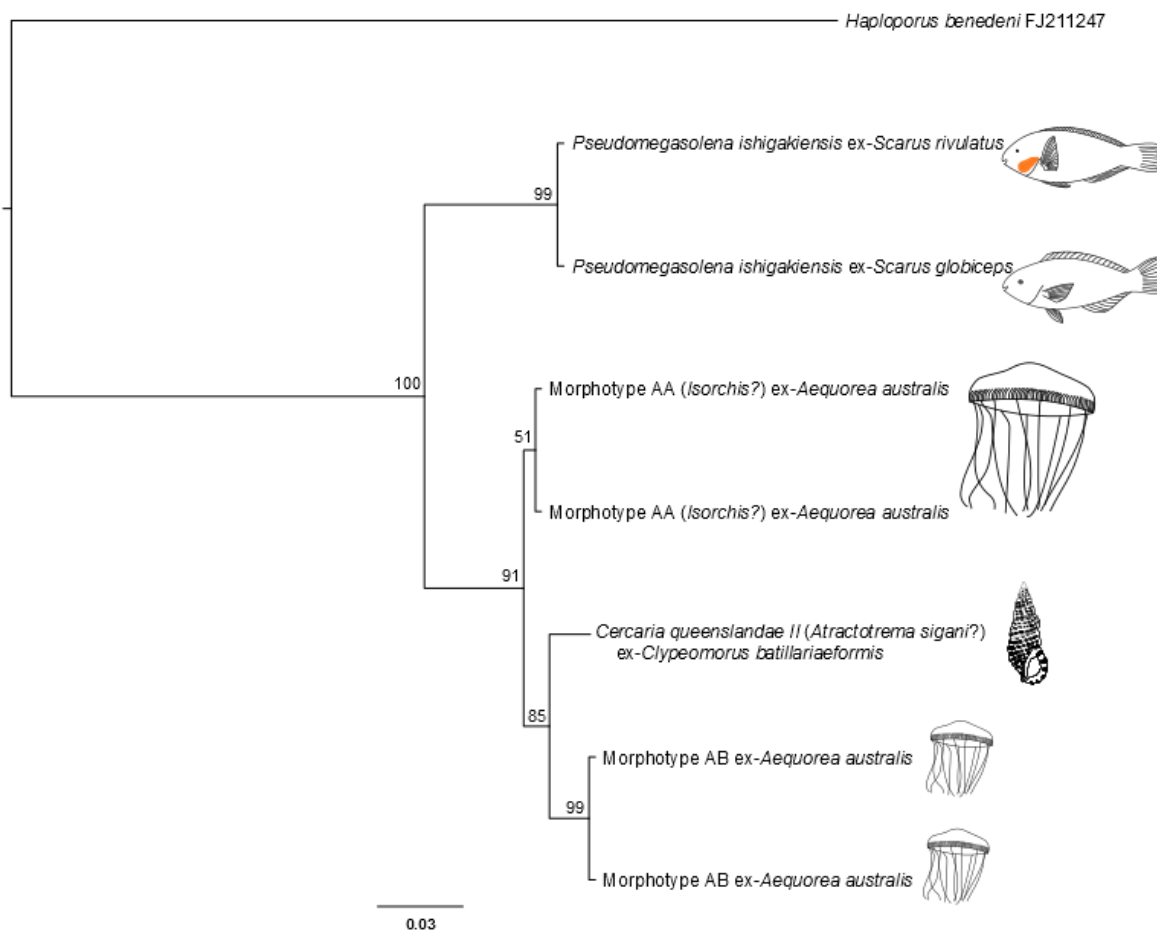


Figure 4-8 Relationships between Atractotrematidae hosted by the hydromedusan *Aequorea australis* (bold) and other atractotrematid taxa based on Bayesian inference analysis of the ITS2 rDNA dataset (employing a K80 + G substitution model). Posterior probabilities are at the nodes, values <50 are not shown. The scale bar indicates branch length (the expected number of substitutions per site)

tentatively identified as a species of *Isorchis*. Comparing the location of Morphotype AB with Morphotype AA in the ITS2 tree (and noting that there are only 6 bp difference), and its proximity to *Pseudomegasolena* rather than *Atractotrema* in the LSU tree, Morphotype AB is tentatively identified as a species of *Isorchis* or *Pseudisorchis*.

4.3.5.3 *Lepocreadioidea* sequences

Two ITS2 sequences were obtained from two lepecreadiid metacercariae (Morphotype LA) hosted by two hydromedusae of the species, *Olindias singularis* from Lizard Island. The sequences were 461 bp long. The entire ITS2 sequence (292 bp) was included and in addition 122 bp of the 3' end of the 5.8S rDNA, and the initial 47 nucleotides of the 5'S end of the 28S rDNA.

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In addition to the Lizard Island lepecreadiid sequences, two sequences were obtained from two lepecreadiid metacercariae from jellyfish collected from Fitzroy Island (Southern GBR). One metacercariae was from an unidentified species of *Beroe* (referred to as *Beroe* sp. B as it differed to the *Beroe* sp. from Lizard Island). The sequence was 463 bp long. It included the entire ITS2 sequence (293 bp), 123 bp of the 3' end of the 5.8S rDNA, and the first 47 nucleotides of the 5'S end of the 28S rDNA. The other lepecreadiid metacercariae was from the hydromedusae *Aequorea australis*. The sequence was 470 bp long. It incorporated the complete ITS2 region (300 nucleotides), 123 bp of the 3' end of the 5.8S rDNA, and the initial 47 nucleotides of the 5'S end of the 28S rDNA.

These lepecreadiid sequences were combined in a Bayesian analysis with the following: *Opechona bacillaris* metacercariae (from *Aequorea eurodina*) and adults (from fish) (Chapter Two), *Cephalolepidapedon warehou* metacercariae (from scyphozoan jellyfish) and adults (from fish) (Chapter Two), *Pseudopisthagonoporus vitellus* metacercariae from *Cassiopea* sp. and an adult from a fish (Chapter Three), a cercaria from a nassariid gastropod (from Leonie Barnett's database), adults from fish (specimens sequenced from Dr T.H. Cribb's collection, and sequences from Dr T.H. Cribb's database) and sequences of adults from fish from Genbank. *Retrovarium sablae* (family Cryptogonimidae) was selected as an outgroup based on the tree constructed by Bray et al. (2009) (Figure 4-9). The analysis consisted of 39 sequences, each consisting of 396 characters. The two sequences from two individual lepecreadiid specimens hosted by *Olindias singularis* (Morphotype LA) were identical. They were not closely related to any other digeneans with the nearest sequence (*P. vitellus*) differing by 39 bp (10%). The sequence from the lepecreadiid hosted by *Beroe* sp. B formed a clade with *O. bacillaris*, differing by only 7 bp (2%). This suggests that the lepecreadiid may be a species of *Opechona*. The ex-*Beroe* sp. B lepecreadiid and *O. bacillaris* formed a well-supported clade with a cercaria from a species of *Nassarius*, differing by 14 to 18 bp (4-5%). The lepecreadiid from *Aequorea australis* (Fitzroy Island) was most closely related to *Diploproctodaeum mamoaafata*, although it differed by 25 bp (6%).

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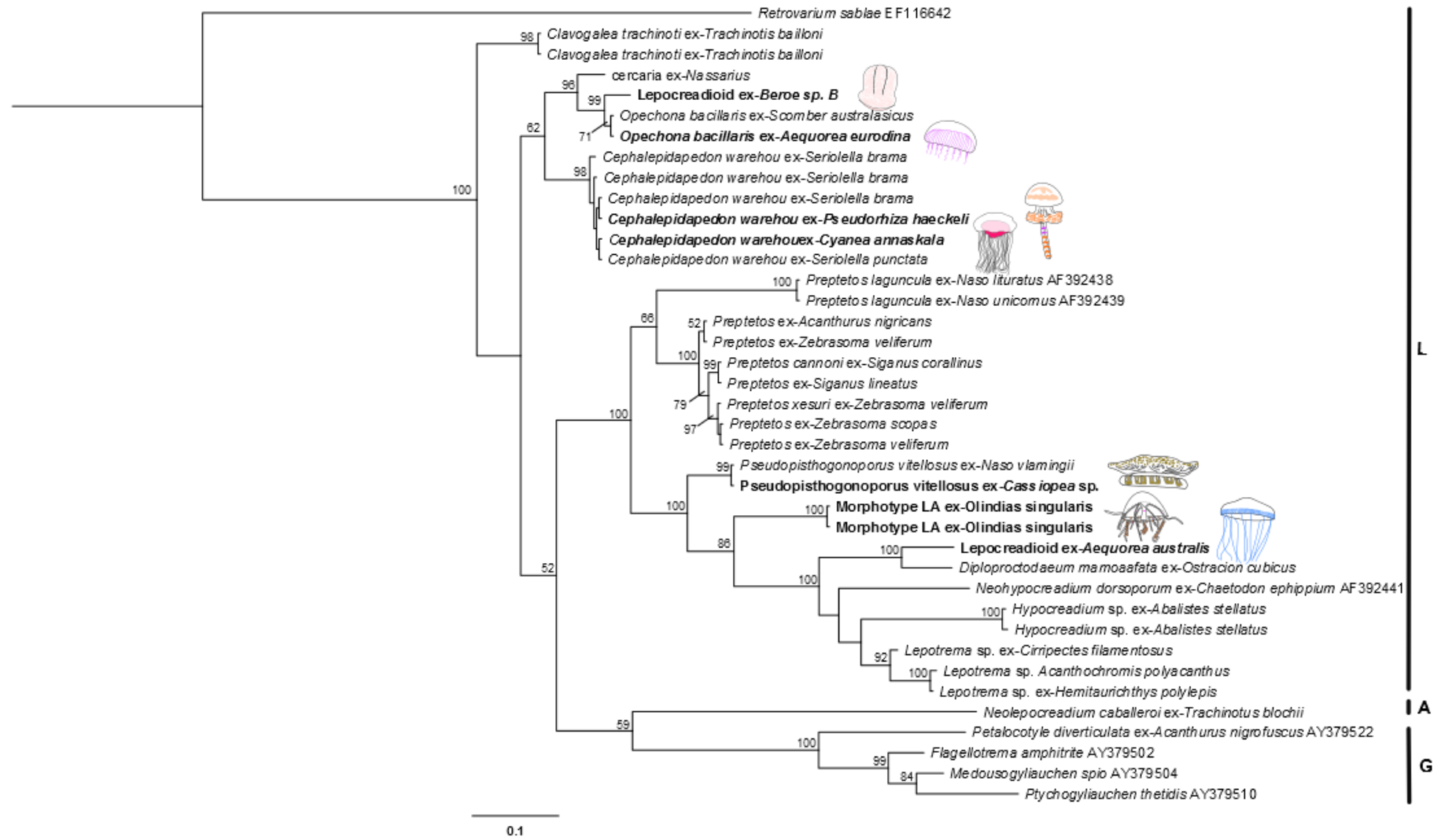


Figure 4-9 Relationships between Lepocreadioidea hosted by jellyfish (bold) and other lepocreadioid taxa, based on Bayesian inference analysis of the ITS2 rDNA dataset (employing a TVM+I+ G substitution model). Posterior probabilities are at the nodes, values <50 are not shown. The scale bar indicates branch length (the expected number of substitutions per site). L = family Lepocreadiidae, A = family Aepnidiogenidae, G = family Glyciauchenidae

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4.3.6 Host specificity

Evidence of host specificity was detected. Didymozoid metacercariae were only obtained from ctenophores (Table 4-3). Molecular results from four specimens suggested that the two didymozoid species were host specific, as each species was found in only one species of host. Of the remaining hemiuroids, *Lecithocladium* sp. was hosted by the hydromedusa *Olindias singularis* and the other (Type HA) by the ctenophore *Bolinopsis* sp. although notably both these hemiuroid species were found only once. Atractotrematids were only recovered from the hydromedusa *Aequorea australis*. Lepocreadioids were found in both ctenophore species and the hydromedusa *A. australis* and *O. singularis*. All lepecreadioid morphotypes occurred in only one host species, except for *Opechona bacillaris* which was hosted by *A. australis* and *Beroe* sp..

4.4 Discussion

Using a combination of molecular and morphological analyses, 16 digenean species were recorded from five ctenophore and hydromedusae host species. This reveals a high diversity of digeneans within relatively few species and specimens of jellyfish hosts. Two species of these digeneans were from the family Atractotrematidae. This is the first record of attractotrematids using a second intermediate host. Using morphology alone, the attractotrematids were initially identified as lepecreadioids but DNA sequencing revealed that they were actually attractotrematids. This demonstrated the importance of using a combination of morphological and molecular analyses in the identification of metacercariae.

4.4.1 Diversity of digeneans using jellyfish as intermediate hosts

This study found a high level of diversity of digenean metacercariae in jellyfish. The only other comparable study of jellyfish digeneans, which investigated metacercariae from multiple jellyfish hosts within one area, found far less diversity. Diaz Briz et al. (2012) recorded only 4 species of digeneans from 50 jellyfish species examined (17 species infected) from the southern Brazilian, Uruguayan and Argentine Continental Shelf. It seems likely that the diversity of digeneans would be greater at Lizard Island, on the Great Barrier Reef, than in the open ocean of the southwestern Atlantic. There is a general increase in diversity of marine organisms towards the tropics (Rohde 1992) and coral reefs are exceptionally diverse environments. Whilst no estimate of the diversity of digeneans in the southwestern Atlantic is available, there are estimated to be 2270 species of digenean infecting fish of the Great Barrier Reef (Cribb et al. 1994). In terms of definitive hosts, the

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diversity of reef fish is estimated at 471 species in the south western Atlantic (Floeter et al. 2008) compared with 1300 species in the Great Barrier Reef (Russell 1983). As parasite diversity is positively correlated with host diversity (e.g. Hechinger and Lafferty 2005), it seems reasonable to assume that diversity of digeneans in host jellyfish would be greater in the GBR than the south western Atlantic. In addition, as Diaz Briz et al. (2012) examined specimens preserved in formalin, only morphological analyses were undertaken, which may have underestimated diversity.

Prevalence varied between different species of digenean, and between different host species. Similar variation was found by Diaz Briz et al. (2012). Prevalence of digenean parasites in jellyfish can be high relative to other zooplankton hosts (Marcogliese 2005a), supporting their importance as digenean hosts. Although replication in the current study was limited, studies with very high replication have recorded high prevalences in jellyfish. For example, 16.8% of the hydromedusan *Clytia hemisphaerica* (956 individuals) were infected by *Monascus filiformis* and/or *Opechona* sp. (Diaz Briz et al. 2012), 29.2% of *Clytia* sp. (n=1670 host jellyfish) were infected by *M. filiformis* (Girola et al. 1992), and 14.7% of 5893 *Pleurobrachia pileus* ctenophores were infected by *Opechona bacillaris* (Yip 1984). As in the current study, two of these studies also found low prevalences of hemiurid species compared with other digenean families (*Hemiuris communis* in 0.8% of 2754 *P. pileus* (Yip 1984); no hemiurids in *C. hemisphaerica* (Diaz Briz et al. 2012); in 9861 *Eucheilota ventricularis* hydromedusae, prevalences of *M. filiformis* and *Opechona* sp. were 7.8% and 5.6% respectively, but hemiurids only 0.02% (Diaz Briz et al. 2012)).

Most of the jellyfish hosted multiple species of digenean parasites. Indeed, four of the five species of jellyfish studied were infected by at least four species of digenean. These findings suggest some jellyfish species may be used as intermediate hosts more frequently, or by more species of parasite, than others. Variation in the diversity of parasites hosted by jellyfish was also found by Diaz Briz et al. (2012), although of the 50 jellyfish species they examined, only two hosted all four digenean species recorded in the study. In the current study, the highest prevalence and intensity of infection were found in the ctenophore, *Beroe* sp. These differences in prevalence in *Beroe* may reflect differences in the trophic ecology of the host jellyfish studied. *Bolinopsis* sp. is likely to prey on small zooplankton (similarly to *Bolinopsis infundibulum* (Costello and Coverdale 1998; Purcell et al. 2010). However, *Beroe* species prey upon other ctenophores, including *Bolinopsis* species (Swanberg 1974; Finenko et al. 2003) and may engulf their prey

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entirely or bite off pieces of prey (Haddock 2007). Thus, parasites in the prey ctenophore may be transferred to *Beroe* sp, a process termed trophic transmission. Trophic transmission has been observed in *Beroe ovata* in the transfer of the parasitic anemone *Edwardsiella lineata* from the ctenophore *Mnemiopsis leidyi* to *B. ovata* when the latter consumes the former (Reitzel et al. 2007), and it seems likely that digeneans are similarly transferred. The diet of *Aequorea australis* and *Olindias singularis* is unknown. *Olindias tenuis* feeds on zooplankton (copepods, chaetognaths, polychaetes, fish larvae and amphipods) (Larson 1986) and *Aequorea victoria* feed on ichthyoplankton, zooplankton and other gelatinous zooplankton (Purcell 2003), so *A. australis* may also potentially bioaccumulate parasites.

4.4.2 Location of parasites within the host

The location of lepopocreadioid metacercariae in the mesoglea of the medusae is consistent with other studies (e.g. *Opechona bacillaris* in *Hydractinea carnea* hydromedusae (Køie 1975), and *O. pyriforme* in *Gonionemus vertens* (Stunkard 1969)). This location corresponds with observations of lepopocreadioid cercariae penetrating jellyfish hosts (Stunkard 1969; Køie 1975). Didymozoids occurred most commonly within the mesoglea or between ctenes within ctene rows. This differed from those infecting the ctenophore *Pleurobrachia pileus* which were mostly attached to the external wall of the pharynx (Yip 1984). The presence of *Lecithocladium* sp. in the pharynx of its host corresponds to the findings of Køie (1991) and Boyle (1966). Similarly, other non-didymozoid hemiurids have been found in the pharynx of ctenophore (Køie 1983; Yip 1984). It is likely that hemiurids infect jellyfish through the consumption of infected crustaceans (see Section 4.4.3) and may remain in the pharynx/stomach or penetrate further into the mesoglea. The attractotrematids and Morphotype LE were encysted within the mesoglea of their hosts. Encysted metacercariae are infrequently recorded from jellyfish hosts with only two recent records (Chapter Three and Martorelli 1996). Some earlier studies (Monticelli 1914; Palombi 1934 and 1937 all cited in Dollfus 1963) reported cysts. These reports were later discounted by Dollfus (1963) who reasoned they could not be encysted, as he identified the digeneans as the non-encysting *Lepocreadium album*. As Dollfus (1963) used only figures to identify the worms, and in light of the current study, Monticelli and Palombi's observations may well be valid. It is not known how attractotrematids infect jellyfish hosts.

4.4.3 Superfamily Hemiuroidea

The complete life cycles of very few didymozoids are known, due to the difficulties of identifying their metacercariae and keeping their definitive hosts (often large pelagic fish) in captivity, which makes feeding experiments impractical (Anderson 1999). Didymozoids are part of the superfamily Hemiuroidea, and the life cycle of this superfamily is generally as follows: sporocysts or rediae develop in gastropods, fork-tailed cystophorous cercariae exit the gastropod host and are eaten by a second intermediate crustacean host where they develop into an unencysted metacercariae, the crustacean is then consumed by a third intermediate host, or directly by the definitive host (Cribb et al. 2003). Therefore, the hemiuroids in this study are likely to be utilising jellyfish as third intermediate hosts.

Lecithocladium sp. and Morphotype HA were located in the gastrovascular systems of their hosts, which would be expected if they had been consumed with their second intermediate host. In addition, another species of *Lecithocladium*, *L. excisum*, appear to use jellyfish as third intermediate host. Cercariae of *L. excisum* infected and successfully developed into metacercariae in the copepod *Acartia tonsa* in laboratory conditions (Køie 1991). In the wild, *L. excisum* is hosted by the hydromedusa *Liriope* sp. and *Muggiaea atlantica* (Reimer et al. 1975), the ctenophore *Pleurobrachia pileus*, the polychaete *Tomopteris helgolandica*, and the fish *Scomber scombrus* and *Trachurus trachurus* (Køie 1991). As these invertebrates feed on copepods, and *S. scombrus* and *T. trachurus* include jellyfish in their diet, it is likely the jellyfish are acting as third intermediate hosts. In the current study, *Olindias singularis*, *Bolinopsis* sp. and *Beroe* sp. are likely to act as third intermediate hosts to their hemiuroid parasites. It is possible *Beroe* sp. may even be a fourth intermediate host, if it has consumed another jellyfish. Morphological and molecular analyses differentiated three didymozoid species, although they cannot yet be identified to genus or species level. The two species which were sequenced were more closely related to didymozoids from scombrids (tuna) (Mladineo et al. 2010) than to those hosted by platycephalids (flathead), a priacanthid (big eye) (Anderson and Barker 1998), haemulids (sweetlips), a sparid (sea bream) and an exocoetid (flying fish) (Abe et al. in press). This suggests the definitive hosts of the jellyfish didymozoids may be pelagic species.

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4.4.4 Family *Atractotrematidae*

A novel finding of this study was the identification of the metacercariae of two attractotrematid species in jellyfish. Indeed, this is the first study to identify any attractotrematid larval stage (Overstreet and Curran 2005). The closely related Haploporidae have a two-host life cycle in which cercariae encyst on algae and are then consumed by fish (e.g. Shameen and Madhavi 1991). Two jellyfish were found to host two species of attractotrematids (one jellyfish was infected by both species, the other only one). One species was closely related to a cercaria, *Cercaria queenslandae* Il. Cannon (1978) suggested *C. queenslandae* Il may be *Atractotrema sigani* based on its Y-shaped bladder and thick spinose tegument so it may be a second species of *Atractotrema*. In the laboratory these cercariae swam actively and then crawled on the substrate and encysted (Cannon 1978). In combination, these findings suggest that attractotrematid cercariae may be able to swim in the water column before penetrating an invertebrate host, as has been shown for lepecoreidiid cercariae (Bray et al. 2009). Sexual adults of *Atractotrema* occur in the intestine of siganids (rabbitfish). Larval siganids feed on plankton (Gundermann et al. 1983) and adults are browsing herbivores whose diet may also include benthic invertebrates such as hydroids (Sano et al. 1984). *Siganus javus* feeds on jellyfish (Baensch and Debelius 1997) and *Siganus fuscescens* has been observed feeding upon the large jellyfish *Catostylus mosaicus* in Moreton Bay (Ben Gilby, pers. comm.).

The second attractotrematid is believed to be a species of *Isorchis*. The definitive hosts of *Isorchis* species include marine and estuarine Mugilidae (mullet) and Chanidae (milkfish), including *Mugil cephalus* (Martin 1973) and *Chanos chanos* (Durio and Manter 1969). Adults of species of *Pseudisorchis* also infect *M. cephalus*. These mullet are planktivores as larvae and early juveniles then switch to consume primarily detritus and benthic microalgae (Whitfield et al. 2012), but also crustaceans, bivalves and fish parts (Blaber 1976; Soyinka 2008). *Chanos chanos* larvae feed on zooplankton, whilst adults and juveniles eat cyanobacteria, algae, small benthic invertebrates (Bagarinao 1994). No second intermediate hosts of any attractotrematids species have previously been recorded (Overstreet and Curran 2005). Jellyfish are not recorded from the diet of mugilid and chanids and thus seem an unlikely second intermediate host. However, as jellyfish are quickly digested by fish (Arai et al. 2003) and thus unrecognized in studies of fish diets, they are often underestimated as a prey item (Mianzan et al. 2001). Therefore, siganids, mugilids and chanids may be infected by attractotrematids by feeding upon infected jellyfish. However, there are several alternative explanations for the infection of

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atractotrematids in host *Aequorea australis*. Firstly, the jellyfish may be accidental hosts. While this is possible, it seems unlikely as the atractotrematids were the most abundant digeneans in the study (13 individuals), and were collected from two medusae on two consecutive days from locations 19 km apart. An alternative is that atractotrematids infect the polyp phase of *A. australis* which could then be incidentally consumed by the fish hosts. If polyps were infected, metacercariae could be retained within medusae as they bud from the polyps. No records of digenean infecting jellyfish polyps have been reported (Browne unpubl.), although polyps are difficult to find and rarely studied in the wild (Willcox et al. 2008). A third alternative may be the consumption of dead jellyfish by the fish. Jellyfish form aggregations (Graham 2001) and may die en-mass sinking to the bottom (Lebrato et al. 2012). Digeneans can remain alive in moribund jellyfish (pers. obs.) so may persist whilst the jellyfish decompose. A final possibility could be that there may be alternative fish hosts of adult atractotrematids that have not been discovered. The discovery of these digeneans in jellyfish hosts suggests a more complicated life cycle may occur than the simple two host life cycle of the closely related Haploporidae. Notably no haploporid metacercariae were found in this study, which, in itself suggests a distinction in the biology of these families. Laboratory experiments and observations of cercariae and potential hosts, and further field collections and associated molecular identification could be used to investigate the life cycle of this family further.

4.4.5 Superfamily *Lepocreadioidea*

The most diverse superfamily in this study was the Lepocreadioidea, with one species (*Opechona bacillaris*) and nine morphotypes identified from Lizard Island, and two molecularly differentiated species from Fitzroy Island. Lepocreadioids generally have a three host life cycle (Cribb et al. 2003). Cercariae emerge from a mollusc host and then penetrate a second intermediate host. The cercariae transform into metacercariae and remain in the second intermediate host until it is eaten by a definitive host (mostly teleost fish). A wide range of invertebrate and vertebrates act as second intermediate hosts, although arthropods are generally an exception (Cribb et al. 2003). Two families within the Lepocreadioidea include jellyfish as second intermediate hosts, the Lepocreadiidae and the Lepidapedidae (Køie 1985 respectively; e.g. Gómez del Prado-Rosas et al. 2000). Therefore, the morphotypes LB, LC, LD, LE, LF, LG, LH and LI may belong to either of these families.

The sequenced lepocreadioids in this study grouped within the family Lepocreadiidae (sensu Bray and Cribb 2012). Lepocreadiids generally have ophthalmotrichocercous

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cercariae which develop in orthogastropod gastropods (Bray and Cribb 2012). Second intermediate hosts include annelids, cnidarians, ctenophores and echinoderms. There are at least eight species of lepecreadiids reported from jellyfish (Browne unpubl.). Therefore it seems likely that *Beroe* sp. B, *Olindias singularis* and *Aequorea australis* are genuine second intermediate hosts for these lepecreadiids. The lepecreadiids from *O. singularis* (morphotype LA) and *A. australis* were unable to be identified further, differing by 25 and 39 bp from the closest species in the Bayesian analysis.

The only lepecreadiid identified to species in this study, *Opechona bacillaris*, has been recorded from 14 jellyfish species previously (Browne unpubl., Chapter Two). These are the first records from *Aequorea australis* and from a species of *Beroe*. Previously, *O. bacillaris* has only been reported from one ctenophore species, *Pleurobrachia pileus* (Lebour 1916; Stunkard 1932; Franc 1951; Fraser 1970; Køie 1975; Yip 1984). The definitive hosts of *O. bacillaris* are predominately fish of the family Scombridae: *Rastrelliger brachysoma*, *Rastrelliger kanagurta*, *Scomber australasicus*, *Scomber japonicus* and *Scomber scombrus* (see Bray and Gibson 1990 for references); but also include fish from 13 other families (see Bray and Gibson 1990). The lepecreadioid from *Beroe* sp. B formed a well-supported clade with *O. bacillaris* (sequenced in Chapter Two) and a cercaria from a gastropod *Nassarius*. It seems likely the metacercariae from *Beroe* sp. B is a species of *Opechona* as it differed by only 7 bp. *Nassarius* species act as first intermediate hosts for *O. bacillaris* and the lepecreadioid *Lepidapedon rachion* (Køie 1975; Køie 1985), supporting the likelihood that the cercaria is closely related to *O. bacillaris*.

4.4.6 The importance of a combined molecular and morphological approach

This study is the first to sequence the metacercariae of didymozoids hosted by jellyfish. DNA sequencing is especially important as a tool for identifying didymozoid metacercariae because the morphological characteristics of didymozoids change significantly as they mature into adults and so morphology is unable to be used for identification of metacercariae (Anderson 1999).

Morphological differences were unable to distinguish any didymozoid metacercariae in the current study, yet molecular analyses were able to distinguish two species. Combined with morphological distinctions of one morphotype, three species were distinguished. Similarly, two atractotrematid species were able to be distinguished using DNA. Indeed, without molecular studies these atractotrematid morphotypes were initially misidentified as

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lepopocreadioids. Morphological techniques alone can underestimate the diversity of digeneans. Studies of jellyfish digeneans using only morphology should, therefore, be viewed with caution. As this has been the only approach used in the past, it may partially account for the relatively small number of digenean species and families known to infect jellyfish, and perhaps explains why atractotrematids have never previously been discovered in jellyfish hosts.

4.4.7 Host specificity

The study indicated most digenean species were host specific, although sample sizes were insufficient to allow firm conclusions. *Opechona bacillaris* was the only species that was found in multiple species of jellyfish. This is unsurprising as *O. bacillaris* has been previously recorded from 14 jellyfish species (Browne unpubl. and Chapter Two). This is the first record of *O. bacillaris* from *Aequorea australis*. In this study didymozoids were only found in the ctenophores, however, they are known from hydromedusae (Reimer et al. 1971; Reimer et al. 1975) as well as ctenophores (Reimer et al. 1971; Reimer et al. 1975; Yip 1984). This is the first record of a didymozoid metacercariae infecting a species of *Bolinopsis*. *Lecithocladium* species have not previously been recorded from *Olindias singularis*. The genus has been recorded from two hydromedusan species (Reimer et al. 1975), and also two ctenophore species (Boyle 1966; Reimer 1976). The atractotrematids were only found in *A. australis*. On two sampling occasions *A. australis* co-occurred with *Beroe* sp. and on one occasion with *Bolinopsis* sp., yet their digenean species were not shared, indicating there may be host specificity in these didymozoid and atractotrematid species. Selection of jellyfish host species has been demonstrated by the cercariae of *Lepocreadium setiferoides*. Under laboratory conditions, the digenean infected *Chrysaora quinquecirrha* but no other “common” species of scyphomedusae or the ctenophore *Mnemiopsis leidyi* (Stunkard 1972). The mechanism by which hosts are selected by digenean cercariae is not known.

4.4.8 Implications of findings

The trematodes of the Great Barrier Reef and adjacent tropical Queensland waters have been extensively studied with 290 fully identified species, and 243 species of trematode-infected fish recorded (Miller et al. 2011). Yet, there are barely any data on species of animals infected with the trematode metacercariae in these waters. This study is one of only eight to identify host species of digenean metacercariae in this region (Chapter Three; Lester and Sewell 1989; Cribb et al. 1996; Beuret et al. 2000; Jones et al. 2004; Miller and

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Cribb 2008; Miller et al. 2009; Grutter et al. 2010) and it is only the second to identify invertebrate hosts of digenean metacercariae in the region (Chapter Three being the first). Information about trematode life cycles, diet and linkages between host species can be used to increase understanding of this tropical reef system. Life cycle information obtained by this study (e.g. the likely use of jellyfish as intermediate hosts by attractotrematids) is also transferable to other systems.

Digeneans which use jellyfish as hosts can infect commercially harvested species of fish and if they influence the health or mortality of their host fish, they may have economic implications. For example, the egg masses of didymozoids in the flesh of heavily infected mackerel *Scomber scombrus* are readily visible and unappealing for consumers. Hosts of attractotrematids, *Chanos chanos*, siganids and *Mugil cephalus* are important aquaculture and commercially fished species. Heavy infections of these parasites could affect the health and value of these fish.

4.4.9 Conclusions

This study detected a diverse digenean fauna from only five jellyfish species. Molecular analyses combined with morphological study proved necessary to fully evaluate the diversity of digenean parasites. Molecular identification was also essential in the discovery of attractotrematids, for which an intermediate host has never been previously found. New host records were identified for *Opechona bacillaris* and didymozoids. The findings of this study provide evidence that jellyfish are important hosts of digeneans.

5 Temporal variation in abundances and life history of the hyperiid amphipod *Hyperia gaudichaudii* and other associates of *Catostylus mosaicus* in Port Phillip Bay, Australia

5.1 Introduction

Many jellyfish form associations with a diverse fauna including parasites and commensals yet ecological data on these associations, particularly time-series, are rare. These fauna include copepods (Browne and Kingsford 2005), shrimp (Martinelli Filho et al. 2008), amphipods (review: Laval 1980), barnacles (Pagès 2000), crabs (Towanda and Thuesen 2006) and fish (Arai 1988). Many associates form commensal relationships, such as fish that derive benefit by seeking shelter amongst the oral arms and tentacles of jellyfish but do not harm the host (Tolley 1987; Kingsford 1993). Such relationships could be important in sustaining recruitment of some species of fish and may be economically important in cases where the adult fish are commercially harvested (Lynam and Brierley 2007).

Parasitic associations, however, can harm the host and may contribute to the demise of jellyfish populations. For example hyperiid amphipods feed on the tissues and organs (e.g. gonads) of their host (Lützen 2005). The amount of damage done may be a factor of the number of hyperiids per host (e.g. Dittrich 1988), the size of the hyperiid (Madin and Harbison 1977) and the regeneration capacity of the host (Laval 1980). On some occasions, the disappearance of a population of medusae has been preceded by a high abundance of amphipods (Dittrich 1988; Mills 1993). Consequently understanding the relationship between gelatinous hosts and their associates may be important for understanding the population dynamics of jellyfish populations and of the animals that associate with them.

Hyperiid amphipods (order Amphipoda: suborder Hyperiidea) are often an important component of the marine pelagic community (Lorz and Pearcy 1975). Some species are highly abundant, for example *Themisto gaudichaudii* is one of the most numerous species of the Antarctic plankton, forming vast concentrations in the surface layer (Vinogradov 1996). Such species were once presumed to be free-living, however, many relationships with jellyfish have been observed and the majority of hyperiids are believed to associate with gelatinous zooplankton for at least part of their life cycle (Harbison et al. 1977; Laval

CHAPTER 5: ASSOCIATIONS OF *CATOSTYLUS MOSAICUS*

1980). *Themisto gaudichaudii*, for example, associates with salps and hydromedusae during its juvenile stage (Sheader and Evans 1975; Madin and Harbison 1977). Hyperiid amphipods are one of the most commonly recorded parasites of gelatinous zooplankton (reviews: Harbison et al. 1977; Madin and Harbison 1977; Thurston 1977; Laval 1980). All reported hosts are gelatinous and include salps, cnidarians, pteropods, heteropods and colonial radiolarians (Harbison et al. 1977; Madin and Harbison 1977). The parasitic amphipods have been proposed to be a polyphyletic group whose structural similarities have evolved through their association with planktonic gelatinous hosts (Lützen 2005). For example, many species have very large compound eyes which may be used to search for and select their hosts (Land 1992); and the mouthparts of divergent species are reduced, apparently modified to consume soft-bodied organisms (Pirlot 1932 cited in Harbison et al. 1977).

Hyperiid amphipods that parasitise jellyfish are important components of the diet of species of diving birds (Bost et al. 1994; Hedd and Montevecchi 2006), fish (Kock et al. 1994; Riascos et al. 2012), squid (Rodhouse et al. 1992), and seals (Nordøy et al. 2008). Because the amphipods feed on the jellyfish prior to being consumed by predators, they may act as an important trophic link between jellyfish and fish (Riascos et al. 2012). Hyperiids also may be a food source for other species that associate with jellyfish. For example, the crab *Cancer gracilis* feeds upon *Hyperia medusarum*, both of which are hosted by the scyphozoan jellyfish *Phacellophora camtschatica* (Towanda and Thuesen 2006).

The life cycles of many hyperiid species are intimately linked with their gelatinous hosts (reviews: Harbison et al. 1977; Thurston 1977; Laval 1980). Generally adult male hyperiids are free swimming and only occur briefly on their host to mate with females (Laval 1980). The females are believed to remain upon the host while brooding their eggs (e.g. Dittrich 1988). Female hyperiids are highly fecund and produce between 10 and 600 eggs (Laval 1980), which they brood within a marsupium (brood pouch). The pre-juvenile stages of some species are completed within the marsupium and once the juvenile stage is reached the female removes the young from the pouch and deposits them on the host, in a process named 'demarsupiation' (Laval 1980). Generally the female swims from host to host depositing one or several larvae upon each. Some species, however, deposit larvae directly within the tissues, or gonads of its host. For example, *Hyperoche mediterranea* digs into the surface of the host ctenophore *Lampetia pancerina* and deposits larvae into the cavity formed (Laval 1980). Eggs, rather than larvae, have been observed being

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deposited into the mesoglea of the leptomedusan *Phialidium* by the hyperiid *Bougisia ornata* (Laval 1980). The eggs hatched after seven days and the larvae of the hyperiid generally remained in or on the gelatinous zooplankton, feeding on its tissues. The larvae of many species are probably obligate parasites because they have hooked dactyls suitable for attaching to their gelatinous hosts, yet they have no swimming appendages (Laval 1980). Once they have developed into adults they may use their hosts as a food source, a resting spot, shelter or transport (Lützen 2005).

Sampling gelatinous hosts and their parasites through time can yield valuable information about the life cycle of the parasite, its population dynamics and the nature of its association with the host. For example, Dittrich (1988) sampled the hyperiid amphipod species, *Hyperia galba*, regularly for two years, and combined with experiments, was able to infer much about the life cycle, host specificity and seasonality of the amphipods. The amphipod infected five species of scyphozoans in the waters around Helgoland in the North Sea from spring to autumn. Intensity and prevalence peaked in September and October, and then by early November the medusae and hyperiids had disappeared. The mean abundance of *H. galba* varied between the host species and also between the two years sampled. In the Danish Isefjord, prevalences of infection of *Aurelia aurita* by *H. galba* similarly reached nearly 100% (Metz 1967). In this four-year sampling period, the timing of the peak and length of time that jellyfish were infected varied between years. In Strangford Lough in Northern Ireland, *H. galba* infected medusae earlier in the year (May to August, reaching 100% prevalence shortly before the disappearance of the medusae (Fleming et al. 2014). While the presence of *H. galba* coincided with the first appearance of medusae in Helgoland (Dittrich 1988), they were not observed on medusae in Strangford Lough until 16-17 weeks after the first appearance of medusae (Fleming et al. 2014). Stable isotope analysis suggested the amphipods had been feeding outside of the host prior to colonising the jellyfish (Fleming et al. 2014). Similarly to Helgoland (Dittrich 1988), jellyfish and *H. galba* were absent over winter in the Isefjord (Metz 1967) and Strangford Lough (Fleming et al. 2014). In Port Phillip Bay, in south-eastern Australia, jellyfish are present throughout the year (Fancett 1986). The hyperiid *Themisto australis* infected *Cyanea* (identified as *C. capillata* but most likely *C. annaskala*; see Dawson 2005a) from winter until spring (July to October) with prevalences highest in August (Condon and Norman 1999). All the *T. australis* observed on the jellyfish were female, whilst in the Isefjord, only a slightly higher number of female to male *H. galba* were present on their hosts (Dittrich 1988).

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The most conspicuous and abundant scyphozoan jellyfish along the east coast of Australia is *Catostylus mosaicus*. Its distribution extends from the Torres Strait to Tasmania (Shepherd and Thomas 1982; Dawson 2005b) and it can form dense aggregations (e.g. 70/30 m³, Kingsford 1993). Associates of *C. mosaicus* include fish (Phillips et al. 1969; Kingsford 1993), spider crabs, shrimp (Coleman 1977), phyllosoma larvae (Thomas 1963), the isopod *Cymodoce gaimardii* (Coleman 1999), the parasitic anemone *Peachia hilli* (Badham 1917), caridean shrimp (Tahera and Kazmi 2006), portunid crabs (Browne and Kingsford 2005), the copepod *Paramacrochiron maximum* (Browne and Kingsford 2005) and the hyperiid amphipod *Hyperia gaudichaudii* (pers. obs.), a species for which almost no ecological information is available. In Port Phillip Bay *C. mosaicus* are often present throughout the year (most prevalent between January to June, Coleman 1999) and its biomass can attain 30,000 tonnes (Hudson et al. 1997). A developmental fishery for the jellyfish was established in Port Philip Bay between 1999 and 2005, although full-scale harvesting never commenced. Impacts caused by parasites of the jellyfish could be high due to the widespread distribution of the jellyfish, its often high biomass and potential use as a food source.

The hyperiid amphipod *Hyperia gaudichaudii* is morphologically identical to the *hystrix* form of *Hyperia medusarum*. They have been considered the same species in the past, however, were separated by Bowman (1973) due to their differing distributions. The distribution of *H. gaudichaudii* is only antiboreal whilst that of *H. medusarum* is boreal, precluding the possibility of gene flow between them (Bowman 1973; Vinogradov 1996; Zeidler 2004). The only previous studies of *H. gaudichaudii* are taxonomic descriptions (e.g. Bowman 1973); measurements of metabolic rates (e.g. Ikeda and Mitchell 1982); records as prey items (e.g. Cherel et al. 2002) and a brief note detailing the occurrence of the amphipod upon several specimens of the ctenophore *Beroë* on one occasion in Port Phillip (Zeidler and Gowlett-Homes 1998). *H. gaudichaudii* also associates with *Desmonema chierchianum* (Browne 1908; Chilton 1912), *Beroë* sp. (Stebbing 1914) and *Chrysaora fulgida* (Barnard 1932).

Ecological studies of the northern hemisphere congener, *Hyperia medusarum* include a shipboard study at two sampling times upon *Chrysaora hysoscella* (Buecher et al. 2001) and a study over five non-consecutive outbreak years of *Phacellophora camtschatica* (Towanda and Thuesen 2006). The amphipods occurred in the gonads, umbrella and stomach pouches of host *C. hysoscella*, and moved from non-gonadal to gonadal tissue as the size of the host increased (Buecher et al. 2001). In some host-parasite relationships,

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the size of the host has been positively correlated with the abundance of parasites (e.g. fish ectoparasites, Lo et al. 1998). There was no relationship, however, between the size of the jellyfish and the number of *H. medusarum* per host. Prevalence was higher in winter (~38%) than summer (~4%). The sex and size of the hyperiids was not measured.

H. medusarum hosted by *P. camtschatica* were concentrated on the oral arms of the medusae, and were not observed burrowing into the bell or gonads (Towanda and Thuesen 2006). Similar to those found on *C. hysoscella*, there was no relationship between the intensity of *H. medusarum* and host *P. camtschatica*. The prevalence of the amphipods varied from ~55 to 100% and were present for the duration that *P. camtschatica* occurred in southern Puget Sound (May until October).

The major objectives of this chapter were:

- To determine temporal variation in prevalence and intensity of ectosymbionts of *Catostylus mosaicus* and to determine whether abundance of ectosymbionts varies with the size of the jellyfish; and
- To examine temporal variation in the location and abundance of the different life history stages of the hyperiid amphipod *Hyperia gaudichaudii* on *C. mosaicus*. Specific hypotheses include:
 - That males are less abundant on *C. mosaicus* than females, except during periods of reproduction;
 - That males occur in the water column as well as on the jellyfish; and
 - That juvenile hyperiids occur only during spring.

5.2 Methods

5.2.1 Collection of specimens

5.2.1.1 Temporal variation in prevalence and intensity of ectosymbionts of *Catostylus mosaicus*

Catostylus mosaicus were sampled nineteen times, at intervals of 3-6 weeks, between August 2008 and September 2010 from the Geelong Arm of Port Phillip Bay (Figure 5-1). On each occasion 20 jellyfish (100-310 mm bell diameter) were collected using a hand held net with an opening of 40 cm and a mesh size of 280 µm. To ensure that the approaching net did not cause symbionts to leave the jellyfish, five jellyfish were viewed underwater by a snorkeler during capture. No symbionts were observed to leave the jellyfish during capture. The jellyfish were weighed using a hand-held scale and the diameter of the bell was measured while the jellyfish was held inverted (Figure 5-2). All

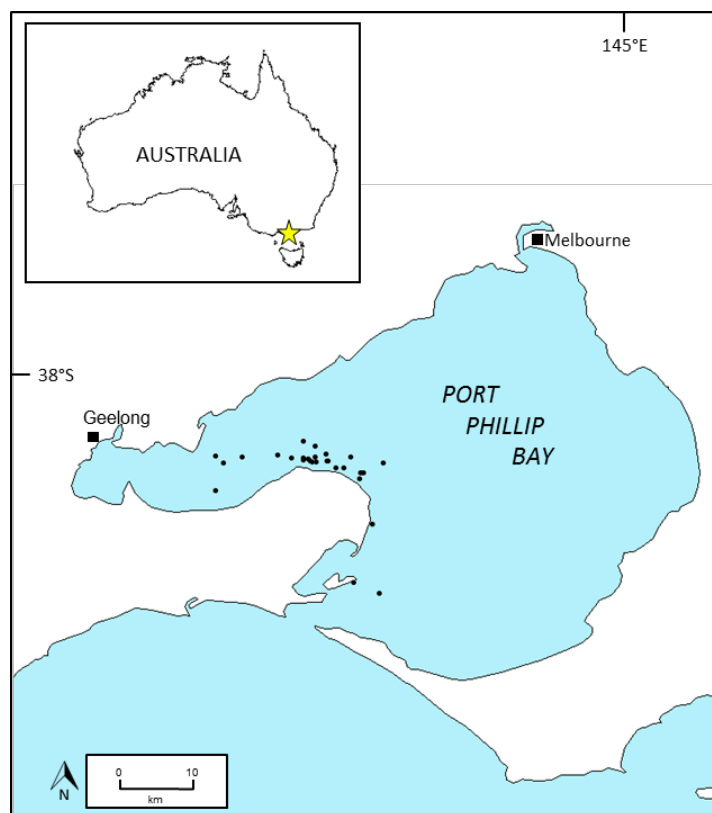


Figure 5-1 Sampling sites (represented by black circles) in Port Phillip Bay, Victoria, Australia



Figure 5-2 Measuring the bell diameter of a jellyfish. Photo: Rod Watson

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isopods, anemones and fish were removed from each jellyfish. Parasitic anemones were preserved in 10% borax-buffered formalin and isopods and fish were preserved in 70% ethanol. The jellyfish were then preserved in 4% borax-buffered formalin diluted with filtered seawater. Water temperature was measured on each occasion.

To increase the number of jellyfish sampled, on five occasions in 2009 (10 March, 1 April, 30 April 2 June, 17 July), when jellyfish occurred in high densities, the size distribution of jellyfish and the prevalence of large symbionts was determined *in situ* whilst snorkelling. The diver swam in a straight line and measured the diameters of 30-100 (average 62) medusae using a ruler. The identity and number of all large symbionts (i.e. those visible to the naked eye, approximately >5mm) on each jellyfish was recorded.

On twelve occasions four of the twenty jellyfish that were within the size range (160- 270 mm BD) were randomly selected and the number, size (length in mm) and location of the different life history stages (male, female, ovigerous female, embedded eggs, embedded juveniles, non-embedded juveniles) of *Hyperia gaudichaudii* on the jellyfish were determined in the laboratory. Only four of the 20 jellyfish were examined because it took 1-5 days to thoroughly examine each jellyfish. The twelve times were selected as occurring at 6-weekly intervals between Sept 2008 and 2010. We attempted to sample jellyfish 15 times in total during this period but on three occasions (6 Nov 2008, 17 Dec 2008, 15 Jan 2009) jellyfish were absent from Port Phillip Bay. Initially each jellyfish was examined under a magnifying lamp and all external amphipods were located and removed. One quarter of the central stomach was then dissected from the jellyfish and examined under a dissecting microscope for the presence of additional amphipods. The central stomach was sub-sampled because the convoluted structure of the gastric cirri meant that searching this area for amphipods was very time consuming. Embedded amphipods were located by cutting the jellyfish into approximately 10 cm sections and examining each section under a dissecting microscope. The position of each embedded amphipod within the jellyfish (oral disc, oral pillars, oral arms, gastric canals, bell, stomach; Figure 5-3) was recorded. Any amphipods that had separated from the jellyfish while preserved and remained in the formalin solution were also recorded and were assumed to be ectosymbionts although their position on the jellyfish could not be determined. The identity, size and location of other symbionts occurring on the jellyfish (mainly isopods, anemones and digenean metacercariae) were also recorded.

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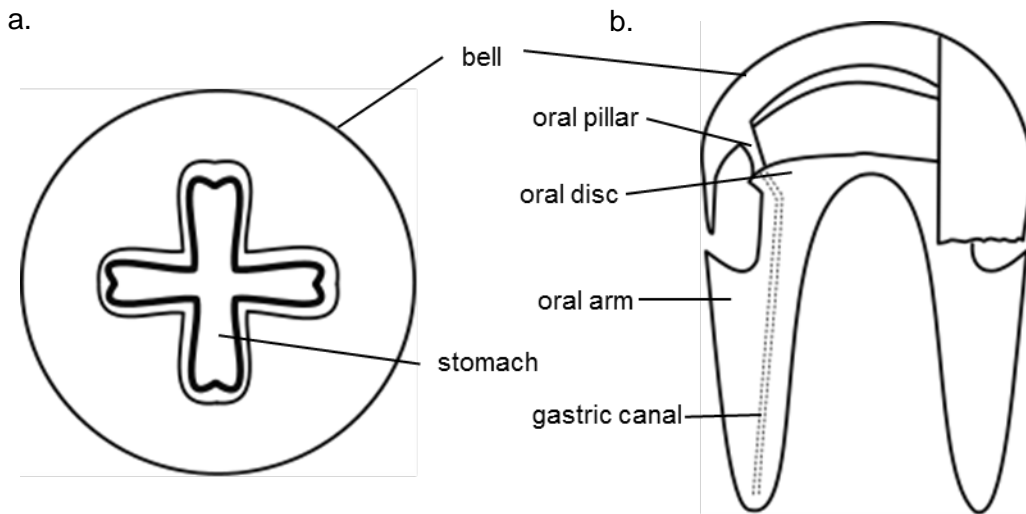


Figure 5-3 Body parts of *Catostylus mosaicus* used in recording position of amphipods (a) subumbrella, (b) longitudinal section. Modified from (Dawson 2005c)

To determine if male amphipods also occurred within the water column, at each time sampled, four vertical plankton hauls were taken within an aggregation of jellyfish and four outside an aggregation (>800 m from jellyfish). The net had a diameter of 50 cm and a mesh size of 250 μm and was lowered to the seafloor and then hauled vertically to the surface. The depth range of the vertical plankton hauls varied from 1.5-14 m (mean of 7.5 m). In the laboratory, plankton samples were searched and the presence or absence of amphipods recorded. The amphipods were identified by Dr Wolfgang Zeidler (South Australian Museum), the isopods by Dr Niel L. Bruce (Queensland Museum), anemones by Assist. Prof. Marymegan Daly (The Ohio State University) and fish by Dr Dianne Bray (Museum Victoria).

5.3 Results

5.3.1 Diversity of symbionts on *Catostylus mosaicus*

Four groups of symbionts were observed on *Catostylus mosaicus*, the sphaeromatid isopod *Cymodoce gaimardii*, the parasitic anemone *Peachia hillii*, fish (*Seriolella brama* and monocanthids including *Acanthaluteres* sp.) and the hyperiid amphipod *Hyperia gaudichaudii* (Figure 5-4). Prevalence of *C. gaimardii* varied throughout the year

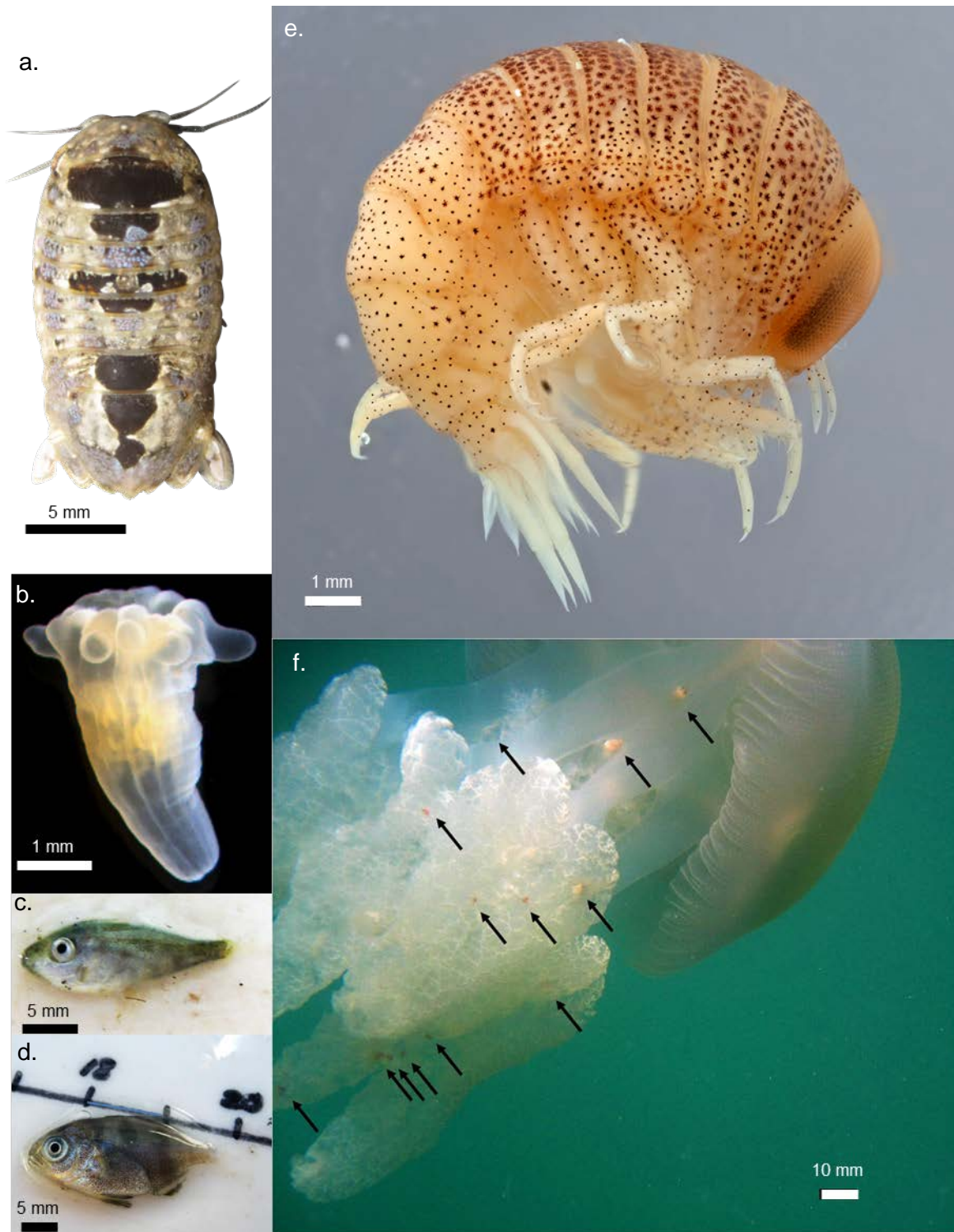


Figure 5-4 Photos of associates of *Catostylus mosaicus* (a) sphaeromatid isopod *Cymodoce gaimardii* (b) parasitic anemone *Peachia hillii*, (c) fish *Acanthaluteres* sp. and (d) *Seriolella brama*, (e) the hyperiid amphipod *Hyperia gaudichaudii* and (f) *H. gaudichaudii* (indicated with arrows) on the oral arms of *C. mosaicus*

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with highest prevalences in autumn 2009 (March: 45%) and summer 2009/2010 (December 2009: 85%; January 2010: 75%; Figure 5-5a). Jellyfish were absent between November 2008 and January 2009, prior to the autumnal peak in the prevalence of *C. gaimardii* in 2009. Isopods were observed on 13 of the 16 occasions that jellyfish were present. Intensity of *C. gaimardii* ranged from 1-5 individuals throughout the sampling period (Figure 5-5b). On several occasions *C. gaimardii* were covered in epibiotic filamentous algae and fine sediment (Figure 5-6). The parasitic anemone *Peachia hilli* had a more restricted temporal distribution and was observed from April to September 2009 and during May and July 2010 (Figure 5-5a). Prevalence ranged from 5% to 20% during these times (mean prevalence of all sampled times was 4.7% and 10.7% during the times that jellyfish were present). Mean intensity was generally low with a maximum mean intensity of 2 ± 1 (Figure 5-5b). Fish were observed in low numbers on four occasions and included the centrolophid *Seriolella brama*, and leatherjackets (family Monacanthidae) including *Acanthaluteres* sp. (either *Acanthaluteres spilomelanurus* or *Acanthaluteres vittiger*) (Figure 5-5a, b).

During the more intensive *in situ* sampling undertaken between 10th March 2009 and 16th July 2009, similar prevalences (Figure 5-7a) and intensities (Figure 5-7b) for large ectosymbionts were found as for the less intensive sampling method described above (Figure 5-5 a, b). The consistency of results between the two sampling methods indicates that the 20 replicate jellyfish provided an adequate representation of the ectosymbiont fauna of *Catostylus mosaicus*.

The abundance of *Cymodoce gaimardii* measured whilst snorkelling was not related to the size (bell diameter) of *Catostylus mosaicus* (Figure 5-8). The maximum intensity of *C. gaimardii* (five) occurred on *C. mosaicus* with a bell diameter of 21 cm and 22 cm and no isopods occurred on jellyfish smaller than 12 cm bell diameter. The isopods occurred on most parts of the jellyfish. They were attached using their pleopods and at times were located in shallow depressions or holes they had presumably excavated in the jellyfish (Figure 5-9, Figure 5-10). Holes (without an associated ectosymbiont) were observed in the bells of 15 jellyfish. Of these 15 jellyfish, three had isopods and three had *Peachia hilli* attached elsewhere. Of the 39 *P. hilli* for which the location upon the jellyfish was recorded, the majority (33 individuals) were on the oral arms, whilst four were inside the bell, and two were attached to the stomach.

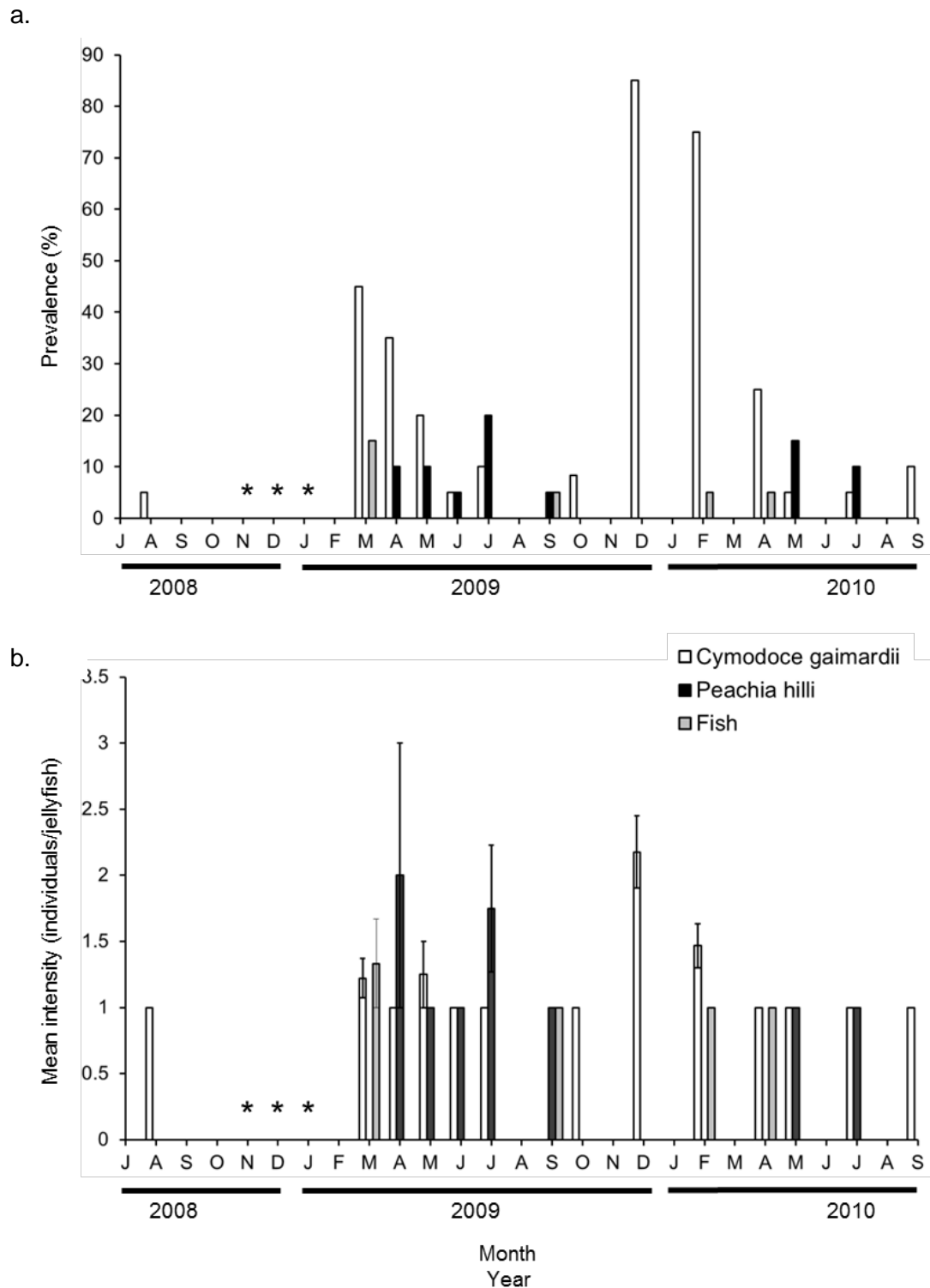


Figure 5-5 Temporal variation in (a) prevalence and (b) mean (\pm SE) intensity of ectosymbionts on *Catostylus mosaicus* (i.e. from the 20 boat samples). * represents sampling occasions when *C. mosaicus* were absent. It should be noted that in September 2008 *C. mosaicus* were present, however, ectosymbionts were not



Figure 5-6 *Cymodoce gaimardii* covered in epibiotic filamentous algae and sediment

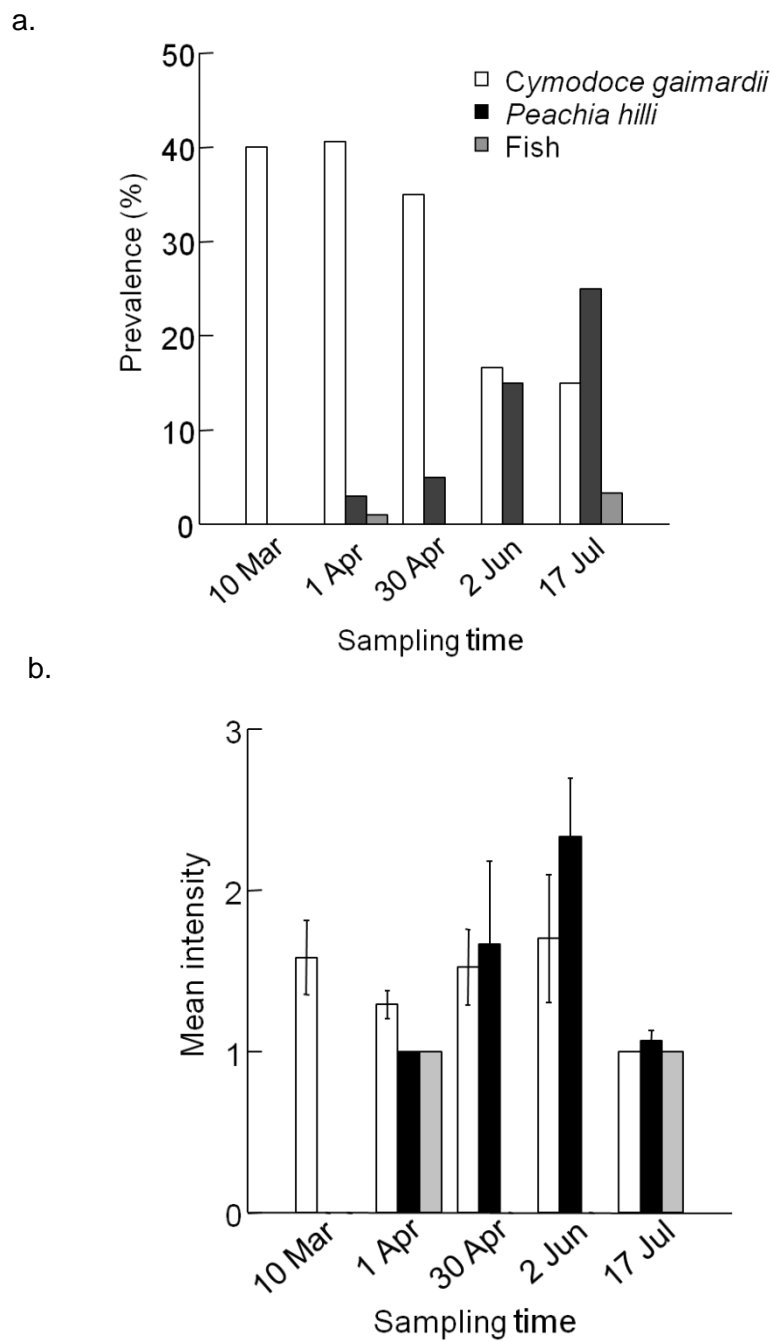


Figure 5-7 (a) Prevalence and (b) mean intensity of large ectoparasites on *Catostylus mosaicus* observed whilst snorkelling

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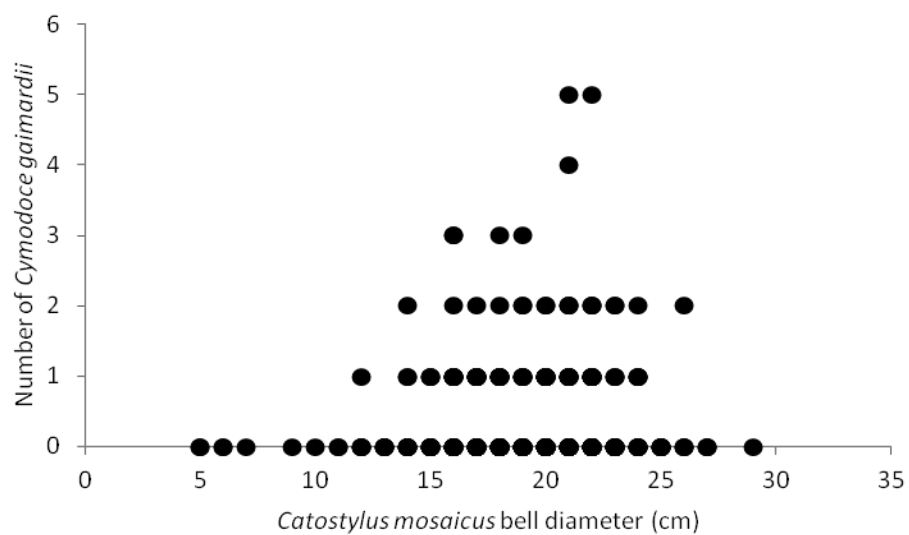


Figure 5-8 Relationship between abundance of ectosymbionts and size (BD (mm) of *C. mosaicus*

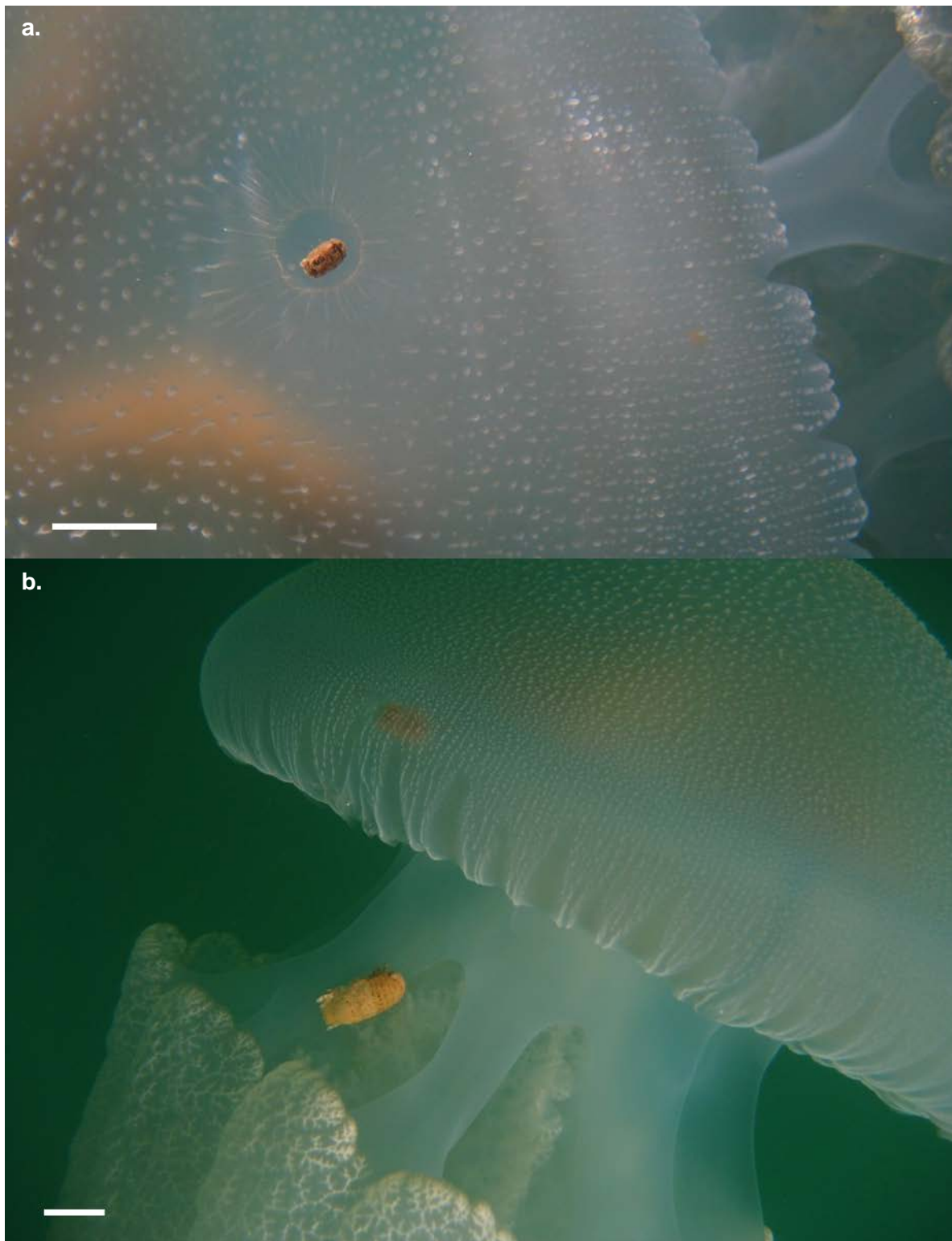


Figure 5-9 Location of *C. gaimardii* isopods *in situ* (a) in shallow depression on bell (b) on oral arms (and exumbrella surface) of *Catostylus mosaicus*. Scale bars represent 3 cm

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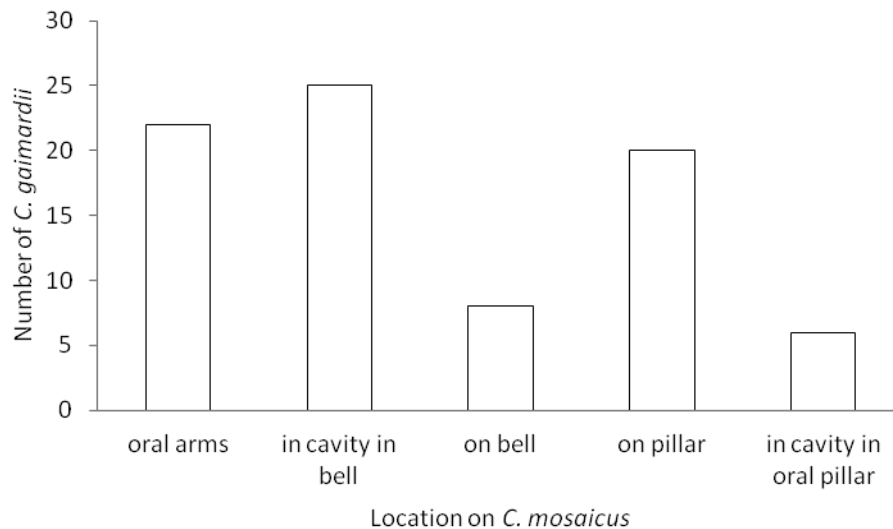


Figure 5-10 Location of *Cymodoce gaimardii* isopods *in situ* on *Catostylus mosaicus*. "In cavity" refers to the isopod being positioned in a shallow depression within the mesoglea of the jellyfish (see Figure 5-9)

Symbionts including *Cymodoce gaimardii*, *Peachia hilli* and digenean trematode metacercariae were recorded on *Catostylus mosaicus* dissected in the laboratory. *C. gaimardii* juveniles, males and females were attached to external surfaces of the jellyfish or had been dislodged after being preserved. One juvenile was inside the stomach of a jellyfish, but had not been digested. Juveniles ranged in size from 5-17 mm (n=9), immature males from 13-23 mm (n=8) and the only female was 15 mm. *Peachia hilli* were located attached to the outside of the oral arms (n=2, length >10 mm), inside the canals of the oral arms at the distal ends (n=2, length=0.7-1.7 mm), in the stomach (n=1, length=12.3 mm) and four had detached after preservation of the host (length=0.70 to 12.3 mm). Fourteen digenean trematodes were dissected from *C. mosaicus*. Intensity ranged from 1 to 2 and prevalence was 8.3%. The trematodes were located primarily in the oral arms (n=8), and also in the stomach (n=4), bell (n=1), and attached to gonadal tissue (n=1). Lengths ranged from 0.18 to 0.43 mm. Two cestodes were embedded in the stomach wall of two individual jellyfish. They were 0.96 to 1.2 mm long.

5.3.2 The association between *Hyperia gaudichaudii* and *Catostylus mosaicus*

All life history stages and both sexes of the hyperiid amphipod *Hyperia gaudichaudii* were recorded on *Catostylus mosaicus* (Figure 5-11, Figure 5-12). All eggs and 21% of juveniles were embedded within the jellyfish. The sex of the amphipods could be distinguished from

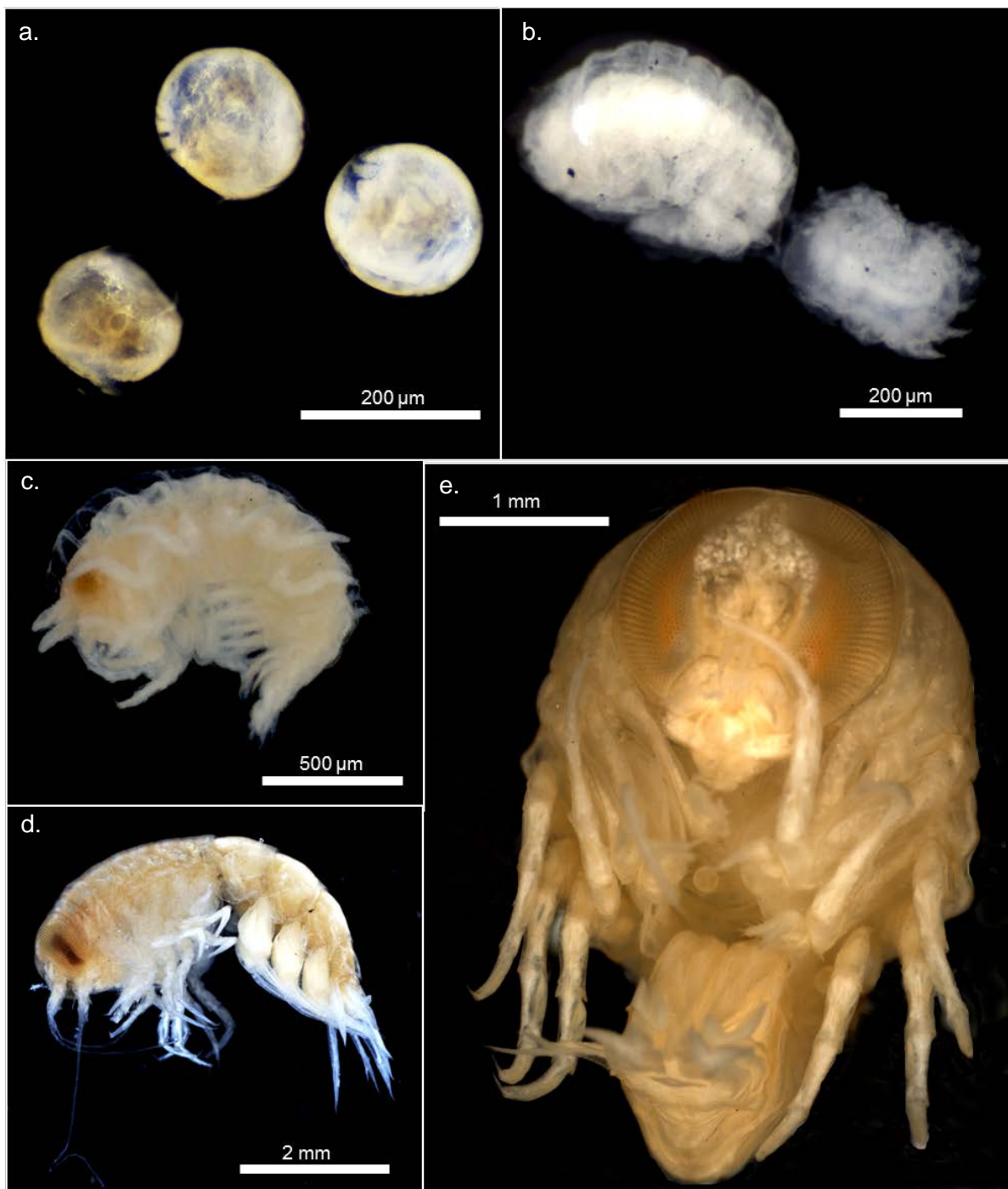


Figure 5-11 *Hyperia gaudichaudii* (a) eggs (b) embedded juveniles (c) non-embedded juvenile (d) male and (e) ovigerous female

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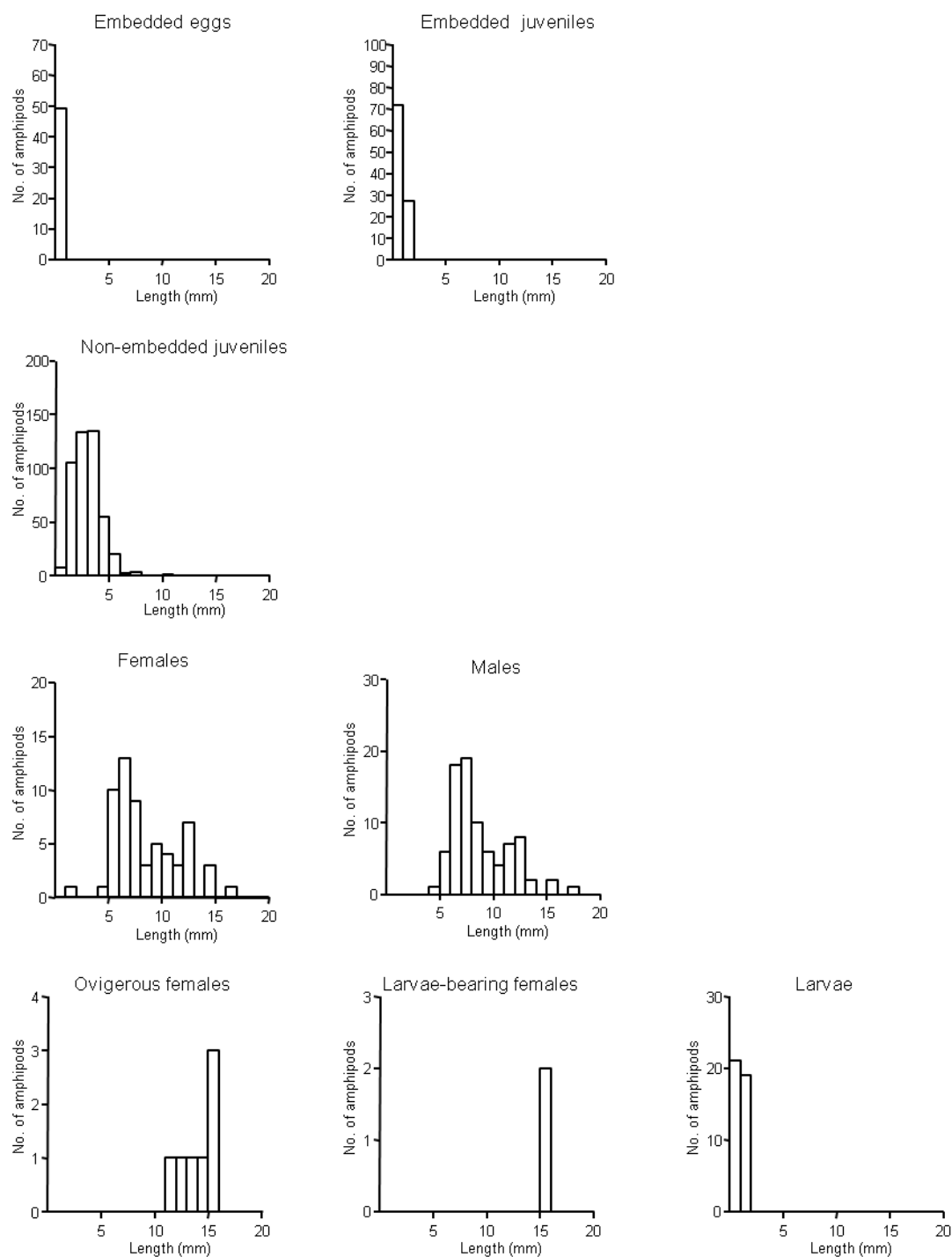


Figure 5-12 Size frequency distribution of the different life history stages of *Hyperia gaudichaudii* sampled from four *Catostylus mosaicus*. Data were pooled across all times sampled

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5 mm upwards. In total, 1131 *H. gaudichaudii* were recorded. Of the 68 females, seven were ovigerous (11-16 mm) and two were larvae-bearing (15-16mm). The minimum number of eggs recorded from one female was 84 and the size of eggs was approximately 0.4 mm (the maximum number of eggs could not be accurately measured as many had become dislodged and were likely to have been lost during sieving).

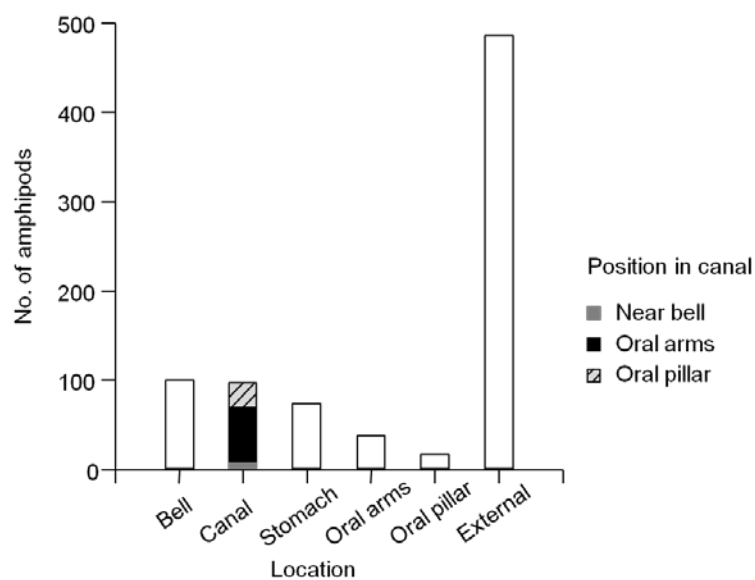
The majority of embedded amphipods occurred in the bell and canals of the jellyfish (Figure 5-13a). Eggs were embedded within the mesoglea of the host (mean depth = $3.75 \text{ mm} \pm 3.54$, $n = 94$). Juveniles were embedded to a maximum depth of 14.0 mm (mean depth = $3.68 \text{ mm} \pm 3.42$, $n = 48$) and most were less than 3 mm, although two larger (7 mm and 10 mm) individuals were observed (Figure 5-13b). The amphipods present in the stomach of the jellyfish (0-10 mm) were intact and had not been digested by the host. Amphipods in the gastrovascular canals of the jellyfish were mostly <5 mm although several individuals from 5 to 11 mm were recorded. All size ranges occurred on the external surfaces of the host jellyfish.

Male and female amphipods co-occurred on the jellyfish in September 2008 and September and October 2009 (Figure 5-14). This observation coincided with the appearance of embedded eggs and juveniles in September 2008 and from September 2009 until May 2010. Three ovigerous and two larvae-bearing females occurred in September 2008; and four ovigerous females occurred in October 2009. Non-embedded juveniles were present in September 2008 and September and October 2009.

The maximum intensities of all non-embedded life stages occurred in September 2008, when 28 females, 51 males and 150 juveniles all occurred on a single host. Moreover, an additional 243 larvae, associated with one female, were present on the jellyfish. This observation occurred immediately prior to the disappearance of *Catostylus mosaicus* within the sampling area. The maximum intensity of embedded eggs (13 individuals host⁻¹) occurred in April 2010 and the maximum intensity for embedded juveniles (14 individuals host⁻¹) in February 2010. The mean abundance of males and females were similar in September 2008 (Table 5-1). On the two other occasions when adults were found, abundances of both sexes were very low. Amphipods were never observed in the plankton samples.

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a.



b.

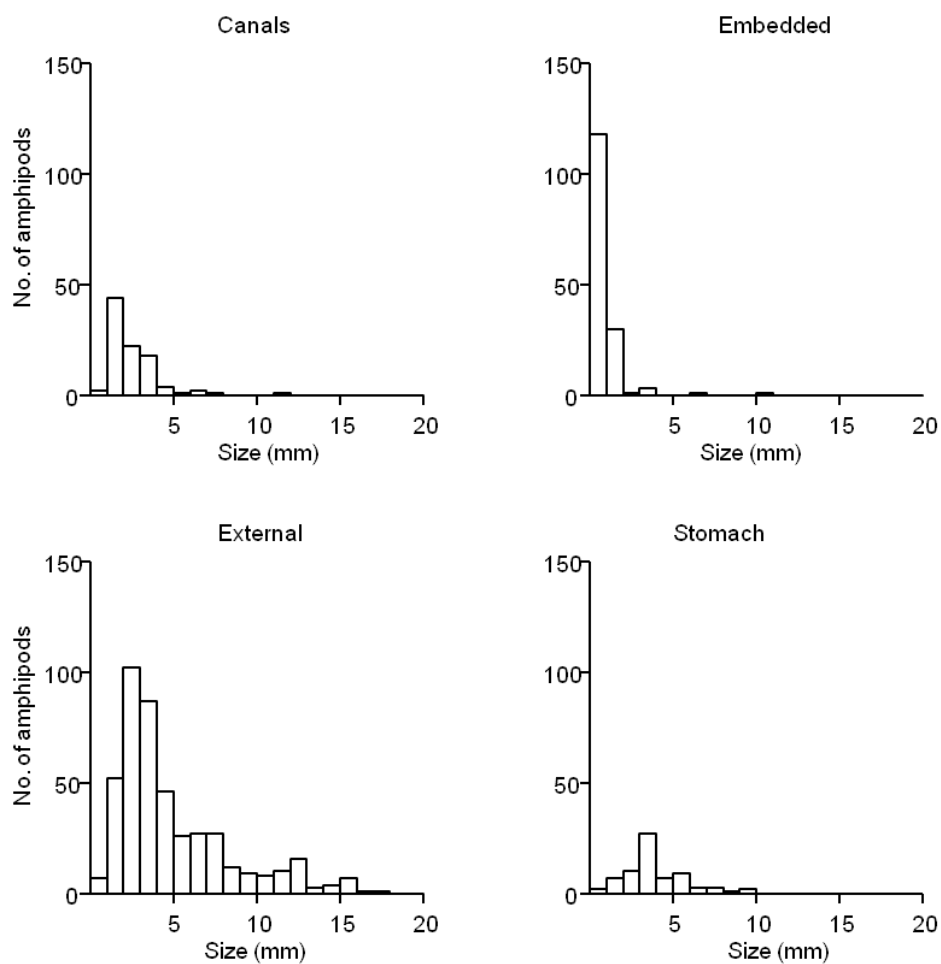


Figure 5-13 Variation in (a) abundance of embedded amphipods among locations within *Catostylus mosaicus* (b) size frequency of external and embedded amphipods in different *C. mosaicus* locations

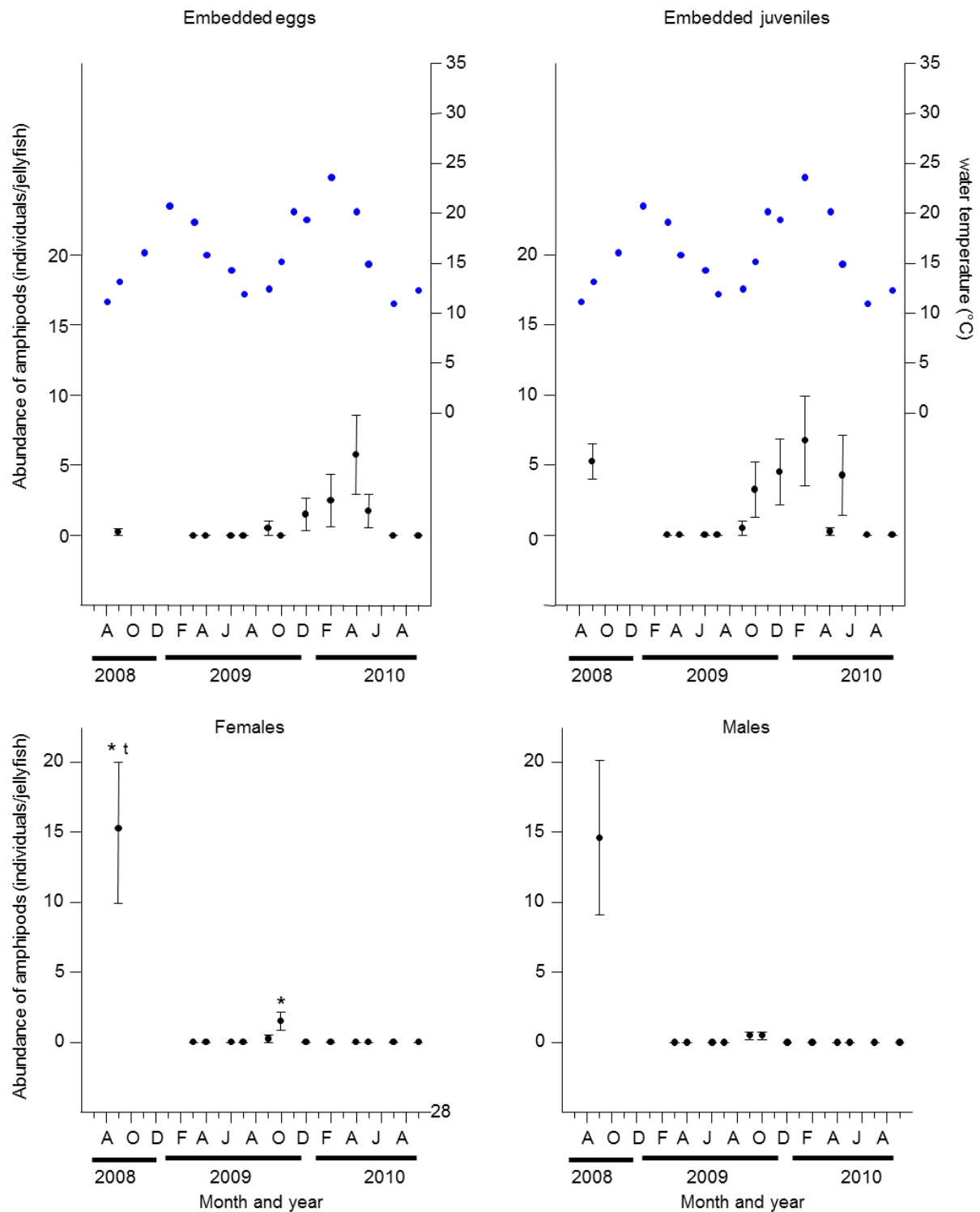


Figure 5-14 Temporal variation in mean (±SE) abundance of each life stage of *Hyperia gaudichaudii* and water temperature. * represents the presence of ovigerous females and ^t larvae-bearing females. Note the change in scale above the break in the y-axis (*cont. next page*)

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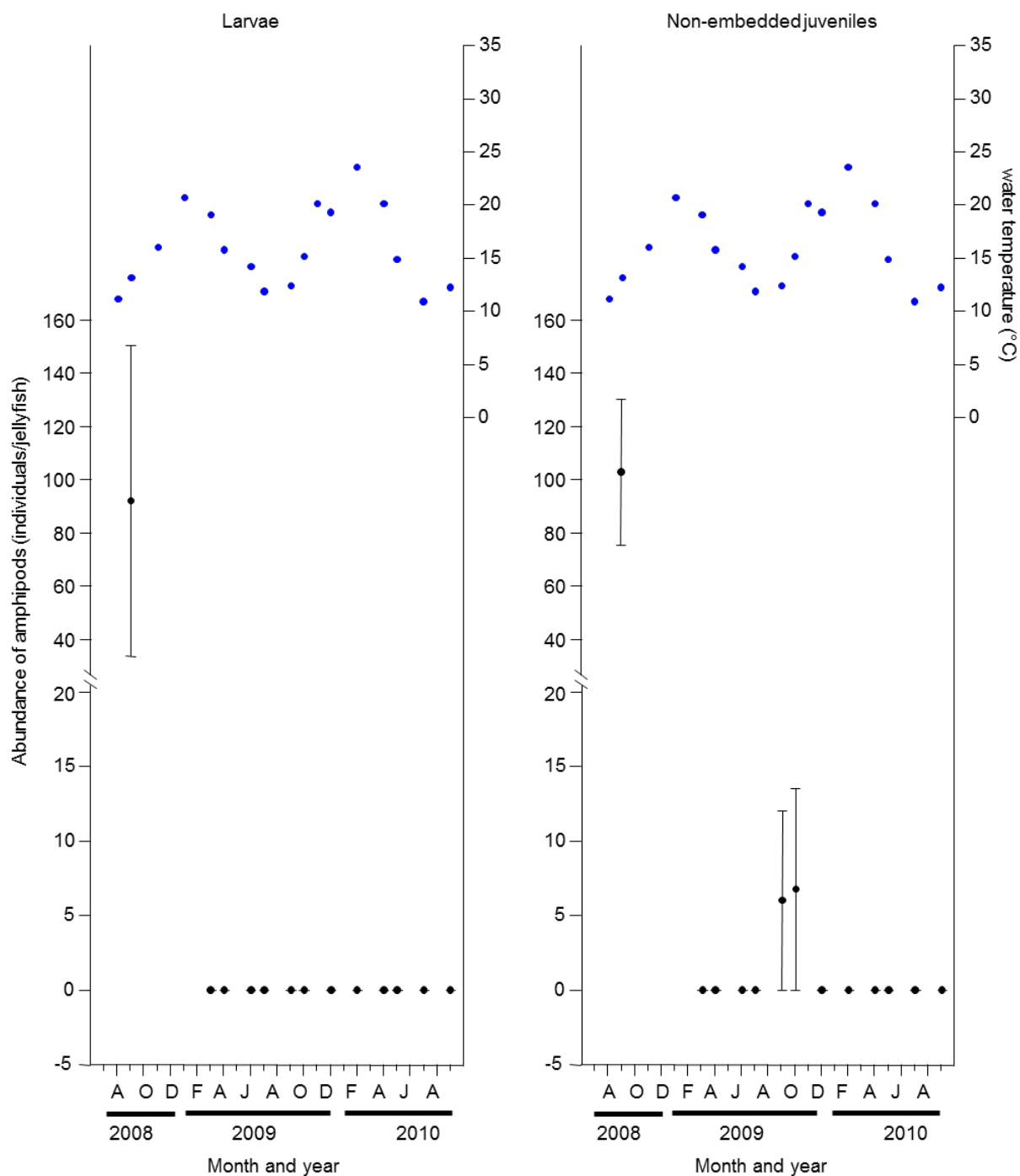


Figure 5-14 *cont.* Temporal variation in mean (\pm SE) abundance of each life stage of *Hyperia gaudichaudii* and water temperature. * represents the presence of ovigerous females and [†] larvae-bearing females. Note the change in scale above the break in the y-axis

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Table 5-1 Mean abundances (\pm SE) of male and female *Hyperia gaudichaudii* at sampling times when present (n=4 host jellyfish)

Date	Male (individuals/host)	Female (individuals/host)
24/09/2008	20.0 \pm 11.0	15.3 \pm 5.3
14/09/2009	0.5 \pm 0.3	0.3 \pm 0.3
21/10/2009	0.5 \pm 0.3	1.5 \pm 0.6

5.4 Discussion

5.4.1 Ectosymbiont communities of *Catostylus mosaicus* in Port Phillip Bay

Ectosymbionts were observed on all occasions that jellyfish were present in Port Phillip Bay and at times prevalences exceeded 80%. Ectosymbionts of *Catostylus mosaicus* were represented by three phyla: the cnidarian *Peachia hilli*, the arthropods *Hyperia gaudichaudii* and *Cymodoce gaimardii* and the chordate fish (including the leatherjacket *Acanthaluteres* sp. and the centrolophid *Seriolella brama*).

The sphaeromatid isopod *Cymodoce gaimardii* occurred on the jellyfish at nearly all of the times that *Catostylus mosaicus* were sampled. The association between *C. gaimardii* and *C. mosaicus* is a novel finding because *C. gaimardii* had previously been considered a benthic species (although they are capable of swimming), having been found in sediments of Port Phillip Bay and Western Port (Victoria), and offshore of NSW (OZCAM 2014). However, *C. mosaicus* has often been observed to encounter the seafloor and then change direction to swim up into water column (pers. obs.) and this behaviour would facilitate the transfer of *C. gaimardii* from the benthos onto the jellyfish. The high prevalences of juvenile and adult isopods on the jellyfish, and the consistency of the association, however, suggests the relationship between *C. gaimardii* and *C. mosaicus* is more than an accidental or brief predatory encounter.

Sphaeromatid isopods have been observed associating with tiger sharks (Newbound and Knott 1999), gastropods (Nishimura 1976), chitons (Glynn 1968), gorgonians (Baker 1926), and mussels (Li 2000). However, apart from a brief note by Coleman (1999) this is only the second record of a sphaeromatid isopod associating with a jellyfish (see also Nogueira Jr and Loyola e Silva 2005) and it is the first study to investigate temporal variability in the infection parameters of a jellyfish-isopod association. Other associations briefly described include a cymothoid isopod with the rhizostome *Mastigias scintillae*

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(Moreira 1961); the giant isopod *Anuropus* associating with *Deepstaria enigmatica* (Barham and Pickwell 1969; Lindsay et al. 2004) and *Stygiomedusa* sp. (Ohtsuka et al. 2010); the idotheid isopod, *Idotea metallica*, associating with the hydrozoan *Velella velella* (Ohtsuka et al. 2010) and the idotheid *Synidotea marplatensis* with the scyphozoans *Chrysaora lactea* and *Lynchorhiza lucerna* and the hydrozoan *Olindias sambaquiensis* (Nogueira Jr and Loyola e Silva 2005).

Unlike the isopod, the parasitic anemone *Peachia hilli* appeared to have a seasonal association with *Catostylus mosaicus* and was only present on the jellyfish between April and September in 2009 and May and July in 2010. The timing of the association observed in the current study was different to that reported by Badham (1917) who found that *P. hilli* only occurred on *C. mosaicus* in Sept and Nov (and rarely in Jan) in Broken Bay, NSW (although the frequency and duration of sampling were not recorded).

Peachia quinquecapitata and *P. hilli* are ingested by jellyfish hosts, feed within the gastrovascular system of the jellyfish and then attach and feed on external surfaces before dropping off to commence a benthic lifestyle (Badham 1917; Spaulding 1972). The location and sizes of *P. hilli* on *C. mosaicus* were similar to those found by Badham (1917), although smaller larvae were recorded in the current study. The ability of the anemone to transfer between jellyfish hosts (pers. obs. and Badham 1917) may account for large holes found on *C. mosaicus* without ectoparasites at the time of sampling (alternatively holes may have been caused by *Cymodoce gaimardii*).

Juvenile fish were observed or captured with *Catostylus mosaicus* on several occasions. These consisted of members of the family Monacanthidae (including *Acanthaluteres* sp.) and the commercially harvested warehou *Seriola lalandi*. These fish associate with jellyfish as juveniles (Last et al. 1983; Kingsford 1993) most likely using them for shelter and protection and, as they are known to feed on gelatinous zooplankton (Kingsford and Milicich 1987; Bulman et al. 2001), possibly as a food source.

5.4.2 The association between *Hyperia gaudichaudii* and *Catostylus mosaicus*

Detailed study of the different life history stages of *Hyperia gaudichaudii* associated with *Catostylus mosaicus* over a period of two years has revealed new information about the relationship between a hyperiid and its jellyfish host. This is the first record of an association between *C. mosaicus* and *H. gaudichaudii* and the first temporal study to dissect *Hyperia* amphipods from within the tissues of a rhizostome jellyfish host, in addition to external surfaces. Embedded eggs and juveniles were observed in Sept 2008 (after

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which jellyfish were absent from Port Phillip Bay until Feb 2009) and from Sept 2009-May 2010. This suggested a protracted period of reproduction from spring to autumn and refuted the hypothesis that juveniles would be present only in spring. Externally located male and female *H. gaudichaudii* (including ovigerous and larvae-bearing females) occurred in large numbers on *C. mosaicus* in September 2008 and in small numbers in September and October 2009. The persistence of embedded eggs through spring-autumn in 2009/2010, despite the absence of adults, may reflect that eggs are laid in early spring and then hatch after varying periods of time. Alternatively, eggs may have been laid continuously throughout spring-autumn and, due to low prevalences of adults and the examination of only four jellyfish each time, sampling may have been inadequate to detect the adults at other times. Lack of amphipods in any of the 301 plankton samples taken inside and outside of aggregations of medusae suggests that the amphipods were not present in large numbers (if at all) in the water column at any of the sampling times. It seems unlikely, therefore, that the adults have a pelagic life style and only associate with the jellyfish to breed. The location of the adults during periods other than spring, therefore, still needs to be resolved.

In many hyperiid species adult males are free-swimming and only associate with host jellyfish to reproduce (Laval 1980). However, males within the genus *Hyperia* may differ to other hyperiids because adult male *Hyperia medusarum* were present on *Phacellophora camtschatica* throughout the time hyperiids and jellyfish associated in Southern Puget Sound (Towanda and Thuesen 2006) as were *Hyperia galba* adult males with *Aurelia aurita* (Dittrich 1988). On the only occasion during which both male and female *Hyperia gaudichaudii* were abundant in the current study (September 2008), the proportion of males and females was similar. Thus the hypothesis that males would be less abundant on *Catostylus mosaicus* than females, except during reproductive periods, was not supported because both males and females were absent from medusae except during spring. During spring, however, males and females occurred in equal abundance on medusae.

Examination of ontogenetic changes in the distribution of the amphipod on the body of the host has allowed us to determine how the amphipod uses the host during its life history. For example, eggs and larvae (<2mm) were embedded in the bell, oral arms and pillars of the jellyfish, indicating that eggs and/or larvae were deposited directly into the jellyfish by the females. Juvenile *Hyperia gaudichaudii* (>2mm) were located in the canals, stomach and external surfaces of *Catostylus mosaicus* but large hyperiids (>10 mm) only occurred

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on the external surfaces of the jellyfish, indicating that individuals may migrate from the gastric region to the external surfaces as they grow. Similar ontogenetic migrations on a host have been observed in *Hyperia galba*, whose larvae and juveniles occur in the gelatinous tissues or radial canals of host *Aurelia aurita* but whose adults occur mostly on the gonads (Metz 1967).

The ontogenetic migration of *Hyperia gaudichaudii* over the body of the host may reflect ontogenetic differences in feeding. Presumably the larvae and juveniles that were embedded in the host feed directly on the mesoglea. However, juveniles were frequently located in the canals and stomach, which are areas where the planktonic prey of the host is concentrated. Although no dietary information on *H. gaudichaudii* is available, diatoms have been recovered from the fecal pellets of the parasitic congener *Hyperia medusarum* (Towanda and Thuesen 2006) and phytoplankton (including diatoms) and tintinnids have been recovered from the guts of numerous species of pelagic hyperiid amphipods (Hopkins 1985). These types of plankton have all been observed in the gut of *Catostylus mosaicus* (Browne and Kingsford 2005) and so may provide a source of food for juvenile amphipods. Adult *H. gaudichaudii* occurred almost exclusively on the external surface of the animal. Other adult amphipods, including *Parathemisto gaudichaudii* (Condon and Norman 1999), *Hyperia galba* (Bowman et al. 1963) and *Hyperia schizogeneios* (Laval 1972) attach to the outer surfaces of their jellyfish hosts with two pereopods and beat their pleopods to apparently filter feed. Similar behavior was observed during the current study when a *H. gaudichaudii* that was attached to the rhizostome *Pseudorhiza haeckeli* was maintained in an aquarium for a several days (pers. obs.), suggesting that adult *H. gaudichaudii* may use *C. mosaicus* as a substrate from which to filter feed on plankton.

Whether juvenile and adult *Hyperia gaudichaudii* also feed on the tissues of *Catostylus mosaicus* is unknown. However, fecal pellets of juvenile, female and male *Hyperia medusarum* collected from the external surfaces of *Phacellophora camtschatica* were almost full of nematocysts (Towanda and Thuesen 2006) and stable isotope analyses of these amphipods indicated that they fed directly on their host. In contrast, *Hyperia schizogeneios* feeds primarily on plankton caught by its medusa host but feeds on the host when planktonic prey are insufficient (Laval 1972). Stable isotope analysis of adult *Hyperia galba* revealed host tissue comprised the greatest component of their diet, although benthic and pelagic components also occurred (Fleming et al. 2014). A dietary study, potentially using the 'pulse chase' approach whereby jellyfish are artificially enriched

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in ^{13}C or ^{15}N and the subsequent incorporation of enriched isotopes into the amphipod measured, should be undertaken to confirm whether *H. gaudichaudii* does derive nutrition from its host.

Parasite populations may regulate the demography of their hosts (Anderson and May 1979; May and Anderson 1979). In September 2008, the prevalence and intensity of *Hyperia gaudichaudii* on *Catostylus mosaicus* was the highest measured during the study. Following this infestation by *H. gaudichaudii*, medusae disappeared from the sampling location and did not reappear until March 2009. When medusae reappeared they were devoid of amphipods and amphipods did not reoccur on the jellyfish until September 2009. By November 2009 the population of jellyfish appeared to decline again. On each occasion that the population of medusae declined the jellyfish appeared to shrink (their bell diameters were smaller), their oral arms appeared to be shorter relative to their bell diameter and small and large holes were present in the bells of many animals. Moreover, in November 2009 the jellyfish were sparse (about one jellyfish was encountered every 1 km) and many dead jellyfish were also observed washed ashore around the bay (pers. obs.). Although the ultimate cause of mortality in the population of *C. mosaicus* in Port Phillip Bay is unknown, high abundances of the hyperiids *Hyperia medusarum* and *Parathemisto pacifica* have been correlated with declines in populations of hydromedusae in Puget Sound, USA (Mills 1993) and it is possible that parasitism by *H. gaudichaudii* may have contributed to the demise of *C. mosaicus* in Port Phillip Bay.

Various hypotheses have been proposed for the persistence of *Hyperia* species in locations despite medusae being absent for extended periods. Dittrich (1988) proposed that *Hyperia galba* overwintered on the benthos of the German Bight area of the North Sea, undergoing hibernation, or possibly on scyphistomae of *Aurelia aurita*. However, Towanda and Thuesen (2006) observed that *Hyperia medusarum* that attempted to rest on scyphistomae in a planktonkreisel tank were captured by the polyps. They therefore proposed the amphipods are likely to overwinter on other medusae species, as they observed in southern Puget Sound. Fleming et al. (2014) argue that the limited jellyfish infestation period (only 5-8 weeks/year) of *H. galba* in Strangford Lough, combined with benthic and pelagic components in their diet (measured by stable isotope analysis) make hibernation an unlikely alternative. Metz (1967) hypothesized that *Hyperia galba* is reintroduced to the small Isefjord (which has no overwintering medusae) each year by the inflow of infected *Cyanea* from the Kattegat Strait, which connects to the North Sea. During the six month period that *Catostylus mosaicus* did not occur in the sampling area,

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the amphipods may have persisted in unsampled areas of Port Phillip Bay on *C. mosaicus*, or other species of jellyfish (e.g. *Pseudorhiza haeckeli* on which high intensities were recorded). Alternatively they may have been reintroduced to the bay on jellyfish swept in from the open ocean.

A limiting factor in this study was the long time required to process each jellyfish for their amphipods. External amphipods could be located and removed fairly quickly (usually <2 h jellyfish⁻¹) but it took 1-5 days to thoroughly search the gastrovascular system (including the canals and stomach) and the internal mesoglea of each jellyfish. However, because nearly 40% of the sampled amphipods were embedded within the mesoglea, or located within the gastrovascular system of the jellyfish, the time-consuming internal search of the jellyfish was warranted. Indeed, this aspect of the study represents a major advance over previous research because it has enabled a more detailed understanding of the life cycle of the amphipod and the nature of its association with its host. In particular, it is now apparent that the amphipods lay their eggs within the tissue of the host and that the juvenile amphipods burrow to the surface of the jellyfish once they have hatched. However, due to the time-consuming nature of the processing, the amount of replication had to be balanced against the duration of the study and frequency of sampling. Thus, it was decided to allocate fewer replicates per sampling time in order to increase the duration of the study. The small number of samples would have contributed to the inability to resolve the location of males and females if they were rare during non-breeding periods. However, whilst the small number of replicates reduced the resolution of the intra-annual variability in the demographics of the amphipods it increased our knowledge of inter-annual variability.

5.5 Conclusions

Catostylus mosaicus in Port Phillip Bay supported numerous symbionts which varied temporally in prevalence and intensity. There was no relationship between the abundance of ectosymbionts and size of the jellyfish. Frequent sampling over two years elucidated some surprising patterns. One of the most novel findings was the almost consistent occurrence of the isopod *Cymodoce gaimardii*, a species previously considered to be benthic, on *C. mosaicus*. Whether the association between *C. gaimardii* and *C. mosaicus* is opportunistic or obligate remains to be resolved. All life stages of the amphipod *Hyperia gaudichaudii* were found within or attached to *C. mosaicus*, and the relationship appears obligatory for at least part of the life cycle of the amphipod. Further research may

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reveal the diet of the hyperiid throughout its life cycle, and more about the movements of the adults.

6 General Discussion

6.1 Introduction

Parasites play important roles in ecosystems and nearly all organisms expend energy avoiding parasites or dealing with their effects (Littlewood 2005). Yet parasites are often overlooked in ecosystem studies, and their importance is underestimated (Marcogliese 2004; Poulin 2004). Parasites can be useful for the study of biological principles such as competition or habitat partitioning (Rohde 1976), can be used as biological tags (e.g. Carballo et al. 2012), and could potentially serve as biological controls (Torchin et al. 2003) and indicators of ecosystem health (Lafferty 1997). Their role within food webs has recently been recognised, and they and may affect the hierarchy (Thompson et al. 2005), stability, chain length (Lafferty et al. 2006) and energy flow (Lafferty et al. 2008) of food webs. Parasites may also regulate the populations of their hosts (Roberts and Dobson 1995).

Jellyfish populations often follow a “boom and bust” pattern that can affect their local environment (e.g. Pitt et al. 2007). Abundances of jellyfish have increased in some regions of the world in recent decades (reviews: Mills 2001; Condon et al. 2013). In addition, some invasive jellyfish species have established populations in new regions (Graham and Bayha 2007). As jellyfish act as hosts for a diverse parasite fauna, the spread of invasive jellyfish and increases in blooms in some regions, may have greater impacts than previously known. Consequently, studying the diversity and population dynamics of jellyfish parasites is essential to assess their importance and effects.

6.1.1 *Summary of major findings*

Prior to this thesis, jellyfish were reported as hosts for 15 named species of digenean trematodes (Browne unpubl.), but because metacercariae (and cercariae) are difficult to identify, there are likely to be more species not yet identified, and most of the parasites' life cycles have not been fully elucidated. A novel aspect of this thesis was the use of DNA sequencing to identify digenean metacercariae hosted by jellyfish (Chapters Two, Three and Four). A combination of sequencing and morphological techniques was used to identify three digeneans to species level (Chapters Two, Three, and Four). The combined methods were also able to distinguish an additional 35 morphotypes of digeneans from jellyfish hosts. Of these, sequences were obtained for nine species. In total, 11 new sequences of jellyfish digeneans were added to Genbank. This will allow the future

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identification of these digeneans, and the elucidation of their life cycles, by researchers worldwide.

By comparing DNA sequences of digenean metacercariae in jellyfish with those of digenean sexual adults occurring in fish, I was able to identify digeneans and also potential intermediate hosts. Sequences of the digenean *Cephalolepidapedon warehou* were obtained for the first time, from adults in two fish species and metacercariae in two jellyfish species (Chapter Two). Previously, *C. warehou* was known from only one fish host, so this was the first discovery of intermediate hosts, and a new definitive host record. As both host fish are medusivorous, a potential partial life cycle was investigated for the first time. The digenean *Opechona bacillaris* was recorded from the jellyfish genus *Aequorea* for the first time (Chapter Two). New host records for *O. bacillaris*, *Aequorea eurodina* (Chapter Two) and *Aequorea australis* (Chapter Four) were obtained. Adult *Opechona bacillaris* from a fish host were sequenced for the first time.

Ecological studies of jellyfish parasites are rare relative to taxonomic and observational studies. DNA sequencing was used in a study of spatial variation in jellyfish parasites for the first time (Chapter Three). This was also the first investigation of endoparasites of a species of *Cassiopea* (Chapter Three). The first record of any intermediate host (the scyphozoan jellyfish *Cassiopea* sp.) for the digenean *Pseudopisthogonoporus vitellus* was obtained (Chapter Three). Sequences were obtained for an additional three species of digeneans from *Cassiopea* sp. and a total of 23 morphotypes were differentiated. Intensities of digeneans were generally low, with the exception of the most prevalent morphotype. A larval cestode infected *Cassiopea* sp. at Lizard Island and Vlasoff Reef. This was one of few records of cestodes infecting jellyfish and the first such record in the Pacific Ocean. The endoparasite communities of *Cassiopea* sp. varied between the four locations. Only two digenean morphotypes, the larval cestode and a commensal copepod occurred at more than one location. The highest diversity of endoparasites of *Cassiopea* sp. occurred at Lizard Island.

The diversity of digenean parasites of a range of jellyfish was investigated in tropical waters for the first time (Chapter Four). A combination of molecular and morphological analyses revealed 16 digenean species from three species of hydromedusae and two ctenophore species. This was a high level of diversity relative to the only other comparable study of multiple digenean species from one area (Diaz Briz et al. 2012). Sequences of didymozoid metacercariae from jellyfish were obtained for the first time, and the first record of a didymozoid trematode in a species of *Bolinopsis* discovered. A new host record

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(hydromedusan *Olindias singularis*) for a *Lecithocladium* species was obtained. Sequencing identified two metacercariae as attractotrematid species. Prior to this thesis, no larval stages belonging to the family Atractotrematidae had been identified in any host group (Overstreet and Curran 2005). In Chapter Four, two species of attractotrematid metacercariae were distinguished; one was believed to be a species of *Isorchis*, the other *Isorchis* or *Pseudisorchis*. This is the first confirmed insight into the life cycle of attractotrematids.

Temporal variation in the relationships between the jellyfish *Catostylus mosaicus* and its associates in Port Phillip Bay was examined for the first time. The isopod *Cymodoce gaimardii*, previously understood to be a free-living benthic species, was consistently found associating with the scyphozoan jellyfish *C. mosaicus* (Chapter Five). This is among the few records of sphaeromatid isopods associating with jellyfish (Coleman 1999; Nogueira Jr and Loyola e Silva 2005). The temporal relationship between *C. mosaicus* and the parasitic anemone *Peachia hilli* was investigated for the first time (Chapter Five). A new host (*C. mosaicus*) for the hyperiid amphipod *H. gaudichaudii* was discovered (Chapter Five). This was the first temporal investigation of the relationship between *Hyperia* amphipods and a jellyfish host, in which endoparasitic eggs and juveniles were included in addition to ectoparasitic life stages (Chapter Five).

6.2 Implications of jellyfish parasites

6.2.1 Jellyfish parasites and fish

The discovery of a large diversity of parasites within jellyfish and the identification of new jellyfish hosts indicates that jellyfish are more important as parasite hosts than previously recognised. In the past, jellyfish were considered to be “dead ends” in pelagic food webs (e.g. Gardner and Howell 1983; Sommer et al. 2002) and only minor components in the diet of fish. The rapid digestion of jellyfish tissue, the difficulty of identifying partially digested jellyfish in studies of gut contents, and assumptions of jellyfish being of low nutritive value all contributed to these beliefs. However, fish are increasingly being identified as regular or occasional predators of jellyfish (Arai 1988; Ates 1988; Mianzan et al. 1996; Mianzan et al. 2001; Arai 2005a; Cardona et al. 2012). The high infection rates of a diverse digenean fauna in the jellyfish sampled suggests that these jellyfish species are likely to be consumed by fish, enabling the digeneans to complete their life cycle. The continuation of a digenean species relies upon the interaction of the hosts within its life cycle. Therefore the presence of a diverse digenean fauna in the jellyfish sampled, suggests most or all of these digenean species rely upon the jellyfish to transfer them to

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their definitive fish hosts, thus emphasizing the importance of these jellyfish in the diet of fish. As digeneans generally have several hosts throughout their life, studying their life cycle can relate these hosts through food, behavior or habitat (Cannon 1978). The presence of a particular digenean species in a jellyfish and a fish may, therefore, reveal a previously unrecognized trophic link. For example, whilst members of the families Mullidae and Chanidae are not recognised as jellyfish predators, the presence of atractotrematids in fish from these families and in the jellyfish *Aequorea australis* (Chapter Four) suggests a trophic link may occur.

6.2.2 *Jellyfish parasites in food webs*

Jellyfish parasites may act as a link between pelagic and benthic food webs, for example when parasitic larval anemones drop from their host to the seafloor, where they mature (Riascos et al. 2013). They may also act as a trophic link between jellyfish and predators (Riascos et al. 2012) when jellyfish parasites become prey. For example, hyperiid amphipods are consumed directly by fish (e.g. Towanda and Thuesen 2006), birds (Harrison 1984) and other organisms (e.g. the crab *Cancer gracilis*, Towanda and Thuesen 2006). In addition to parasites which are specifically targeted by predators, parasites may be consumed inadvertently when jellyfish are consumed. In such an instance, heavily parasitised jellyfish would presumably increase the nutritional value of host jellyfish, thus increasing the benefit to jellyfish predators. This has not yet been examined for jellyfish, although it has been shown for other hosts. For example, cestode-infected brine shrimp exhibited twice the triglyceride concentration of uninfected brine shrimp (Sánchez et al. 2009). The free-living stages of parasites are often numerous and may also be significant energy resource (Johnson et al. 2010). For example, cercariae of the jellyfish parasite, *Opechona bacillaris* are consumed by the hydroid and medusae phases of *Podocoryne carnea* (Køie 1975).

6.2.3 *Jellyfish parasites and blooms of jellyfish*

There is evidence that in some regions around the world jellyfish populations are increasing (e.g. Sea of Japan), whilst in others they are decreasing (e.g. Northwest Bering Sea) (Uye and Ueta 2004; Brodeur et al. 2008; Condon et al. 2013). If parasite abundances are increased by jellyfish blooms this could increase transmission to other hosts in the parasite's life cycle. Parasite abundances and species richness in first intermediate hosts has been positively correlated with abundances and species richness in final bird hosts (Hechinger and Lafferty 2005). Therefore it seems likely that increased

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abundance and diversity of parasites utilising jellyfish blooms could lead to increased abundance and diversity of parasites in definitive hosts such as fish. Worldwide, the abundance of fish has been reduced by fishing (Jackson et al. 2001; Myers and Worm 2003). Areas with increased jellyfish abundances and decreased fish abundances may result in an increased prevalence of parasites in fish. This could, however, be counterbalanced by the reduction in parasites expected from exploited fish stocks (Dobson and May 1987). Reductions in densities of fish can reduce the prevalence of indirectly transmitted parasites (Amundsen and Kristoffersen 1990). Regions in which abundances of both fish and jellyfish decrease may also decrease in parasite abundances.

6.2.4 Socio-economic effects of jellyfish parasites

Several commercially important species of fish are definitive hosts of jellyfish parasites. Examples include blue, Atlantic and chub mackerel which are commonly infected by digeneans most likely transmitted by jellyfish (Korotaeva 1974; K  ie 1991; Cremona and Sardella 1997; Bartoli and Bray 2004; Chapter One). The effects of these digeneans on their fish hosts have not been measured, although prevalences can reach up to 100% (Bray and Gibson 1990). Most digenean families which infect jellyfish are small gastrointestinal parasites as sexual adults and may be relatively benign in adult fish. However, all parasites reduce the fitness of their hosts in some way (e.g. Bartoli and Boudouresque 2007) and may have a commercial impact, for example by reducing fish condition (e.g. Lemly and Esch 1984) or affecting the appearance of the flesh (e.g. didymozoids, Pascual et al. 2006; Chapter Four). In juvenile and larval fish the effects may be greater due to the larger size of the parasites relative to their hosts. For example, a species of *Opechona* (probably *Opechona bacillaris* according to K  ie 1975) has been found in 0-group haddock *Melanogrammus aeglefinus* (MacKenzie 1974) and may contribute to mortalities of the haddock (MacKenzie 1974). Another parasite which uses jellyfish as an intermediate host and has documented effects on commercial fish species is the nematode *Hysterothylacium aduncum* (Gaevskaya et al. 2002). Infections in larval fish can be lethal in commercially important species (e.g. Rosenthal 1967; Balbuena et al. 2000). There is also a record of human infection, most likely from eating raw fish (Yagi et al. 1996). There are few records of nematodes in jellyfish; however, it is uncertain whether this is due to low prevalences and rarity or because they are overlooked by investigators.

Aquaculture farmed fish may also be affected by jellyfish digeneans. The monacanthid *Thamnaconus modestus* is cultured in Japan (Nagasawa et al. 2011) and Korea (Cho et al. 2001). This species is parasitised by the lepecreidiid *Lepotrema clavatum* (Bray and

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Cribb 1996) in the wild. As this species is medusivorous (Masuda et al. 2008), *T. modestus* may be infected by feeding upon parasitised jellyfish (e.g. *Aurelia* sp., Ohtsuka et al. 2010). Jellyfish should be considered as sources of parasitic infection to fish in sea cages, such as *T. modestus* in risk analyses. While no jellyfish digenean parasites are yet understood to be a problem, the scyphozoan *Pelagia noctiluca* harbours the bacterial fish pathogen *Tenacibaculum maritimum* and has been linked to mortalities of sea-caged fish (Delannoy et al. 2011).

6.3 Directions for future research

Much research on jellyfish endoparasites has examined small species of jellyfish which are transparent and, therefore, easy and quick to sample (e.g. Yip 1984; Diaz Briz et al. 2012). The current study differed from others by investigating large (e.g. *Catostylus mosaicus*, Chapter Five) and opaque (*Cassiopea* sp., Chapter Three; and *C. mosaicus*, Chapter Five) scyphozoans. The use of these jellyfish resulted in extensive dissection times, which limited the number of replicate jellyfish that could be examined. Rapid sampling to detect the presence and abundance of parasite-infected animals can be undertaken using species-specific probes in real-time PCR assays or loop-mediated isothermal amplification (LAMP). For example, metacercariae of the oriental liver fluke, *Clonorchis sinensis* has been detected in fish muscle using a LAMP assay (Cai et al. 2010). Such techniques could potentially be used to detect and quantify parasite species within jellyfish tissue. Species-specific probes in real-time PCR assays have also been used to accurately identify and quantify four larval crustacean species in plankton samples (Pan et al. 2008). This technique could be useful in scanning plankton samples for pelagic stages of jellyfish parasites, for example, hyperiid amphipods and the metacercarial stage of digeneans. As real-time PCR becomes increasingly cheaper, such methods may prove to become quicker and more cost-efficient than traditional dissecting/sample sorting methodology. This may be especially advantageous for larger jellyfish, which can be more problematic when occurring in large blooms, for example the giant jellyfish *Nemopilema nomurai* (Uye 2014).

Jellyfish are major prey for charismatic marine fauna such as ocean sunfish (MacGinitie and MacGinitie 1968; Bigelow and Schroeder 1974) and turtles (e.g. Heaslip et al. 2012). Five turtle species which feed upon jellyfish (Shaver 1991; Bjørndal 1997; Brand-Gardner et al. 1999; Polovina et al. 2000; Blumenthal et al. 2009; Heaslip et al. 2012) range from vulnerable to critically endangered on the IUCN Red List of threatened species (Marine Turtle Specialist Group 1996; Seminoff 2004; Abreu-Grobois and Plotkin 2008;

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Mortimer and Donnelly 2008; Wallace et al. 2013). Little is known about the life cycles of parasites infecting turtles (e.g. pronoccephalid digenean trematodes) and jellyfish should be considered as potential intermediate hosts. This may be particularly relevant for leatherback turtles which feed predominately on pelagic medusae (review: Eckert et al. 2012). Ocean sunfish are parasitised by many species. *Mola mola*, for example, hosts 54 species of parasites, including cestodes, monogeneans, digeneans, copepods, isopods, cirripedes, branchiurans, nematodes, protozoans and acanthocephalans (Love and Moser 1983). Jellyfish are probably intermediate hosts for trophically transmitted sunfish parasites, particularly didymozoid trematodes (Purcell and Arai 2001; Chapter Four). Didymozoids can attain lengths of 12 m in sunfish and may affect the health of the fish (Noble 1975). Sequences from only three species of metazoan parasites that infect sunfish species (a cestode, a monogenean, and an accacoeliid digenean) are available on Genbank (Olson et al. 2001; Olson et al. 2003; Perkins et al. 2009). Further sequencing of sunfish parasites may well match the sequences from jellyfish obtained in this, or future studies.

At least 13 species of jellyfish are recognized as invasive (see Mills 2001; Purcell et al. 2007 and references within; Frost et al. 2010; Gravili et al. 2013). Parasites may spread with their hosts when hosts invade new areas, infecting native communities (Prenter et al. 2004). Alternatively, if parasites from the host's native range do not reach the invaded area, the host may be released from negative consequences of parasites, which may facilitate their proliferation in the new area. Parasites native to the invaded area may also infect invasive hosts, and then “spillback” into the native community (Kelly et al. 2009). Invasive hosts and their parasites may also affect the food webs of the invaded area (Britton 2013). Despite the importance of invasive jellyfish, very few studies have examined their parasites. For example, in the Black Sea, the invasive ctenophores *Mnemiopsis leidyi*, *Beroe ovata* and *Pleurobrachia pileus* were infected by larval stages of the nematode *Hysterothylacium aduncum*. The hydromedusa *Olindias singularis* is infected by digenean metacercariae in its native waters (Chapter Four) but there has been no investigation of its parasites in its invasive location, the Mediterranean Sea (Gravili et al. 2013). Studies of invasive jellyfish should include investigations into the presence and abundances of parasites.

Jellyfish exhibit classic ‘boom and bust’ population dynamics. Blooms can appear rapidly due to their rapid growth rates (e.g. Pitt et al. 2013) and ability to reproduce in large numbers when conditions are favourable (e.g. Brodeur 1998). In addition, jellyfish blooms

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may be short-lived and are often seasonal (Mills 2001; Pitt et al. 2014). These characteristics are likely to have a significant impact upon the relationships of jellyfish and their parasites and warrant further investigation. Changes in the phenology of blooms (for example, the shifting of ctenophore blooms by one month in Cheseapeake Bay; Condon and Steinberg 2008), their frequency, and magnitude may significantly affect parasite populations. For example, digenean cercariae emerge from their first intermediate hosts in great numbers, from several dozens to hundreds of thousands depending on the species of digenean and factors including host size and age, and water temperature (Galaktionov and Dobrovolskij 2003). The asexual production of such large numbers of cercariae resulting from a single initial infection, contributes to the evolutionary success of digeneans (Cribb 2005). The many cercariae produced presumably compensate for the difficulty of finding a host in the short period available (generally 24-48 hours, see Pechenik and Fried 1995). If large numbers of jellyfish were present during such critical periods they would presumably be infected in great numbers, increasing the overall transmission success of the digenean. Conversely, changes in parasite populations may affect their host populations. For example, the survival of larval hyperiids is temperature dependent (e.g. Dittrich 1987) and likely to be a primary influence on the distribution of hyperiid species. *Hyperia gaudichaudii*, for example, is a cold water species (Vinogradov 1996) and although common in Port Phillip Bay (Chapter Five), is not recorded from the warmer waters of eastern Australia, despite the abundance of its host *Catostylus mosaicus* (Shepherd and Thomas 1982; Pitt and Kingsford 2000; Dawson 2005c). Climatic changes could, therefore, affect populations of *H. gaudichaudii* and thus potentially its host, *C. mosaicus*.

There has been a substantial amount of research on the factors influencing the production of jellyfish blooms, yet relatively little on what causes their collapse (Pitt et al. 2014). Mensurative studies have linked declines of jellyfish populations to increases in abundances of parasites (e.g. Yip 1984; Mills 1993) but it is unknown whether there is a causal link. Experiments in which loads of parasites are manipulated could determine the effects of parasites (at different intensities) on jellyfish hosts, and potentially on jellyfish host populations.

6.4 Conclusion

The primary objective of this thesis was to increase understanding about jellyfish parasites and their interactions with hosts in the marine environment. Much of the research about jellyfish parasites has thus far been descriptive; to increase knowledge of the wider

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implications of jellyfish parasites, more experimental studies are now required. The contribution of this thesis is the observational work that can be used to develop hypotheses that can be tested experimentally. Advances in DNA sequencing have allowed the confident identification of parasite species, without labour-intensive feeding experiments. The findings and further development of techniques in this thesis will facilitate continued discoveries.

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